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RESEARCH PAPERS

VOLATILE CARBONYL COMPOUNDS IN STORED DRY WHOLE MILK¹

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SUMMARY

Flavor constituents in the low-temperature vacuum distillate of reconstituted dry whole milks were largely carbonyl in nature. The 2,4-dinitrophenylhydrazone derivatives identified by paper and column chromatography, ultraviolet studies, and melting points revealed qualitative differences in the milks studied. The following were conclusively or tentatively identified in the distillate from an average dry whole milk: C₃ through C₇, C₈, C₁₁, and C₁₅ n-alk-2-ones; C₁, C₂, and C₉ alkanals and *cis*- and *trans*-furfural. In contrast, a badly deteriorated powder yielded: C₃, C₄, C₆, C₁₁, and C₁₅ n-alk-2-ones; C₁ through C₃, C₅ through C₇, C₉, C₁₀, and C₁₂ n-alkanals; benzaldehyde, two mono- and two diunsaturated carbonyls. The poor powder could be distinguished from the average dry milk mainly by the large number and concentrations of aldehydes it contained.

The relative amounts of individual carbonyls in four additional dry whole milks manufactured and stored under various conditions were determined. The results suggest that the complex problem of organoleptically characterizing stale and oxidized flavor deterioration in dry whole milk stems in part from the large numbers and variable quantitative relationships of the carbonyl compounds involved.

The absence of methyl ketones in dry whole milk prepared with deodorized milk fat suggests a potential means of increasing the storage life of the product. Ketone formation appears to be an important deteriorative mechanism in stored dairy products containing milk fat.

PART I. RECOVERY AND IDENTIFICATION OF CARBONYLS

The use of dry whole milk for beverage purposes has been hindered by its inadequate dispersibility in water and its tendency to develop off-flavors that become increasingly objectionable during storage. Although progress has been made in improving dispersibility, the flavor problem has not yielded appreciably to concerted applied research. The fact that prevention of fat oxidation, and associated oxidized flavor, does not satisfactorily solve this problem has been generally recognized. A so-called stale flavor seems to evolve in the best of dry whole milks. The earlier work of Whitney *et al.* (14) contains observations on such a defect. A need for additional fundamental information on this problem, particularly with reference to the flavor compounds involved, served as the impetus for this study.

EXPERIMENTAL PROCEDURE

Collection of volatiles. The dry whole milk was reconstituted one part of powder to five parts of distilled water. This product was vacuum-distilled for 3 hr. at 35-40° C. and 15-20 mm. Hg in a 5-gal. Pyrex carboy, according to the

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procedure employed by Day *et al.* (5) and in a later study by Wong *et al.* (15). Preliminary observations on the distillate trapped by means of a wet ice and two dry ice-ethanol traps indicated that flavor and aroma in the trapped distillate were due mainly to carbonyl compounds. The odor of the distillate was dissipated upon the addition of 2,4-dinitrophenylhydrazine³ reagent, but was not materially influenced by the addition of acid or base. On the basis of these results, the carbonyl compounds in a relatively good dry whole milk and a decidedly stale, oxidized product, as determined by an experienced taste panel, were investigated to determine the differences in their carbonyl components. Twenty-five pounds of each product was employed in this investigation, necessitating five individual vacuum distillations on each powder.

Preparation of DNP hydrazones. To the distillate from reconstituted dry whole milk was added an acid solution of DNP hydrazine reagent (2 g. of 2,4-dinitrophenylhydrazine per liter of 30% H_2SO_4). The distillate-reagent mixtures from all three traps were combined and held at 40° F. for seven days (2). The DNP hydrazones were extracted with carbonyl-free hexane and the extract dried with Na_2SO_4 . The solution then was filtered and evaporated under vacuum to a volume suitable for column chromatographic separations.

Chromatographic separations of DNP hydrazones. Initial separation of the DNP hydrazones was accomplished by the column partition chromatography methods A or B of Day *et al.* (4). Despite the merits of these procedures, they lack the ability to separate mixtures of DNP hydrazones with the following formulas: Alk-2-one_N , alkanal_{N+1} , alk-2-enal_{N+2} , $\text{alk-2,4-dienal}_{N+4}$, where N is equal to the number of carbon atoms in the ketone. Therefore, when it was apparent that mixtures of DNP hydrazones were present in the bands separating on the partition column, pure derivatives were obtained in many cases by collecting the forepart of the fraction or numerous small fractions from each band. In certain instances, further separations of mixtures from the partition column were accomplished by the adsorption chromatography method of Gordon *et al.* (7).

During the latter stages of the investigation the paper chromatographic method of Gaddis and Ellis (6) was published, which method succeeds in the separation of the previously mentioned DNP hydrazone mixtures. The tentative identification of some higher molecular weight carbonyls was effected with their procedure.

Identification of DNP hydrazones. Tentative identifications of the DNP hydrazones were accomplished by comparing retention volumes of the unknown derivatives on the column partition chromatograms with those reported by Day *et al.* (4) for known derivatives. Rf values by paper chromatography also were used for this purpose, i.e., the method of Huelin (8) for carbonyls up to eight carbons in chain length and the method of Klein and de Jong (11) for carbonyls of higher molecular weight. Both procedures were used in conjunction with ultraviolet spectral analysis in 95% ethanol and in 0.25 N NaOH in ethanol according to the reports of Braude and Jones (1) and Jones *et al.*

³ 2,4-Dinitrophenyl is abbreviated DNP throughout.

(9). The use of alcoholic NaOH was especially effective in differentiating between aldehyde and ketone DNP hydrazones. Conclusive identifications were obtained by melting point and mixed melting points with authentic DNP hydrazones on a Fischer micro-melting point apparatus.

RESULTS AND DISCUSSION

Identification data for carbonyl compounds from the good and deteriorated dry whole milks are given in Tables 1 and 2, respectively.

TABLE 1

Spectral and melting point data in the identification of DNP hydrazones of carbonyl compounds from a relatively good dry whole milk

DNP ^a hydrazone	Absorption maximum (m μ)		Melting point (° C.)		
	Observed	Reported	Observed	Authentic	Mixed
<i>Cis</i> -furfural	369	379	230-235	232
<i>Trans</i> -furfural	375	386
Formaldehyde	345	346	165	166	166
Acetaldehyde	356	356	157	157	157
Acetone	362	363	126.5	127	126.5
Butanone-2	360	362	105.5	116	106
Pentanone-2	362	362	143	143	143
Hexanone-2	360	362	106	106	106
Heptanone-2	362	362	71- 72	72	72
Nonanal	358	358	102
Nonanone-2	362	362	37	38	38
Undecanone-2	360	362
Pentadecanone-2	360	362

^a Tentative identifications based on data from paper and column chromatograms.

TABLE 2

Spectral and melting point data in the identification of DNP hydrazones of carbonyl compounds from a deteriorated dry whole milk

DNP ^a hydrazone	Absorption maximum (m μ)		Melting point (° C.)		
	Observed	Reported	Observed	Authentic	Mixed
Formaldehyde	345	346	165	166	166
Benzaldehyde	378	378	237	238	238
Acetaldehyde	356	356	157	157	157
Acetone	362	363	126	127	126.5
Propanal	358	358	152	149-152	150-152
Butanone-2	360	362	116.5	116	114
Pentanal	358	358	98	99	98
Hexanal	356	358	106	106	106
Heptanal	358	358	106	104	104
Octanal	358	358	105	106	105
Nonanal	358	358	103	102	102.5
Nonanone-2	362	362	38
Decanal	358	358
Undecanone-2	362	362
Dodecanal	358	358
Pentadecanone-2	362	362
Unidentified	375	88
Unidentified	390	186
Unidentified	388-390	168
Unidentified	377	160-162

^a Tentative identifications based on data from paper and column chromatograms.

The presence of methyl ketones in these products was not unexpected, since they have been reported previously (15) in dairy products undergoing substantial heat treatments. The precise origin of these compounds in milk fat, however, is not known at this time.

The results of this study indicate that fat oxidation had been initiated in the relatively good dry whole milk and had progressed to a greater extent in the deteriorated product. Saturated and unsaturated aldehydes are known products of lipid autoxidation. Tentative identification of nonanal as the only higher aldehyde in the good powder does not preclude the existence of other aldehydes. Failure to detect such compounds may be attributed to low concentrations and the lack of adequate methods of separation for various aldehyde-ketone DNP hydrazone mixtures as previously mentioned. Although data usually sufficient for conclusive identification were obtained on four unsaturated carbonyls in the case of the deteriorated product, the results could not be correlated with those for any known unsaturated carbonyls. Regeneration of the derivatives in question with one part H_2SO_4 and one part water resulted in odors suggesting the presence of unsaturated aldehydes.

The flavor characteristics of the two dry milks used in this investigation suggest that the differences lie in the type and concentration of carbonyls present. The deteriorated product had by far the largest concentration and variety of carbonyls. Whereas the relatively good dry whole milk was characterized mainly by the presence of methyl ketones, the deteriorated product contained both methyl ketones and saturated and unsaturated aldehydes. This suggests that differences in flavor of the products can be attributed to the aldehyde content of the powders. However, the term relatively good, used to describe the flavor characteristic of the better powder, indicates that it had a typical dry whole milk flavor. In this connection, it must be remembered that conventional dry whole milk at its best is not generally acceptable for beverage purposes from a flavor standpoint. Thus, the possibility that methyl ketones may contribute to the normal flavor of dry whole milk needs to be considered.

PART II. RELATION OF CARBONYL CONCENTRATIONS TO FLAVOR QUALITY

Part I of this report has shown that volatile carbonyl compounds are involved in flavor deterioration of stored dry whole milk. The second part of the investigation was undertaken to determine the type and relative concentrations of carbonyls in dry whole milks manufactured and stored under various conditions. Flavor studies on the reconstituted products before and after vacuum distillation and flavor evaluations of the distillates from these products were also conducted to establish whether any correlation exists between the off-flavors of the products and the type and quantities of volatile carbonyls they contain.

EXPERIMENTAL PROCEDURE

Samples: The quantitative determinations of this investigation were conducted on four samples of dry whole milk packed and stored under the following conditions:

Sample A—Packed in an atmosphere of nitrogen and stored at 40° F. for a period of 6 mo.

Sample B—Packed in an atmosphere of nitrogen and held at 40° F. for 27 mo.

Sample C—Vacuum-packed and held 3 mo. at 100° F., followed by further storage at 40° F. for 3 yr.

Sample D—Prepared with deodorized milk fat and stored for 9 mo. at 70° F. in an atmosphere of nitrogen.

A sample of pasteurized, homogenized milk was included as the control sample in this study. Ten pounds of Samples A, B, and C; 5 lb. of Sample D, and 4.85 gal. (5.0 lb. of milk solids) of the fresh product were employed.

Collection of volatile carbonyls and preparation of 2,4-DNP hydrazones. The collection of volatile carbonyls and their conversion to 2,4-DNP hydrazones were accomplished according to procedures reported in Part I. The DNP hydrazones were separated initially by the chromatographic methods A or B of Day *et al.* (4), employing 40-50 g. of celite. The forerun to the first band eluted from the columns was rechromatographed by Method A or the method of Corbin *et al.* (3), to isolate DNP hydrazones not observed on the column in the initial separation.

Each individual fraction separated by the column chromatographic procedures was divided into two equal parts. Tentative identification of the DNP hydrazones by ultraviolet studies and paper chromatography, using the methods of Gaddis and Ellis (6), Klein and de Jong (11), and Huelin (8), was performed on one part of the original fraction. The remaining part was used for quantitative studies.

Quantitative studies. For quantitative evaluation, the fractions were evaporated to dryness and redissolved in a definite volume of purified hexane or 95% ethanol. An accurately measured sample was streaked on filter paper and chromatographed either by the method of Gaddis and Ellis or that of Huelin. The separated bands were cut from the paper, extracted with 95% ethanol, filtered, and evaporated to dryness under vacuum. The DNP hydrazone residue was taken up in a definite volume of 95% ethanol and the optical density obtained at the wavelength of maximum absorption (previously determined by the qualitative studies). In general, the optical densities of the DNP hydrazones of aldehydes were made at 358 $m\mu$, of ketones at 363 $m\mu$, and of monounsaturated aldehydes at 375 $m\mu$. Formaldehyde and acetaldehyde DNP hydrazones were analyzed at 345 and 356 $m\mu$, respectively. Calculations for determining the concentrations of carbonyls in the various products were made as described by Wong *et al.* (15).

Flavor studies. Prior to distillation of the products, 10 g. of each was reconstituted with 80 ml. of distilled water. These samples, the distilled milks adjusted to normal total solids and the distillates, were evaluated by a taste panel of three experienced observers. Table 3 presents the flavor observations on these samples, together with the types of carbonyls found in the dry milks.

TABLE 3

Flavor and odor qualities of reconstituted dry whole milks before and after distillation as related to the types of carbonyl compounds detected in the distillates

Sample	Storage conditions	Flavor of product prior to vacuum distillation	Odor characteristics of distillate	Flavor of product after vacuum distillation	Type of carbonyls recovered
A	Nitrogen-packed Held 6 mo. at 40° F.	Rough, lactone, cooked	Cooked, cheesy, buttery, waxy, reminiscent of staleness	Rough, lactone, flat	Ketones
B	Nitrogen-packed Held 27 mo. at 40° F.	Typically stale, slightly oxidized	Old milk, aldehyde-like, oxidized, pungent	Flat, reasonably good product	Ketones, aldehydes, monounsaturated aldehydes
C	Vacuum-packed Held 3 mo. at 100° F. + 3 yr. at 40° F.	Stale, coconut, oxidized	Old milk, old fat, ketone-like	Flavor improved somewhat	Ketones
D	Nitrogen-packed, deodorized milk fat Held 9 mo. at 70° F.	Oxidized, stale	Old fat	Mild, musty, waxy, flat	Low molecular weight ketones aldehydes, unsaturated aldehydes
Pasteurized, homogenized fresh milk	Normal milk flavor	Reminiscent of aldehydes	Flat	Low molecular weight ketones and aldehydes

RESULTS AND DISCUSSION

Concentrations of carbonyl compounds recorded in Table 4 are not absolute, quantitative data. Vacuum distillation under the conditions of this study does not allow for quantitative recoveries of volatiles from reconstituted dry whole milk. Moreover, it has been reported that the yields of carbonyls with DNP hydrazine differ between carbonyls (2, 15). However, details of the vacuum distillation and reaction period between carbonyls and reagent were standardized to eliminate any differences in the yields other than the actual differences in the products themselves. Thus, the concentrations listed in Table 4 are considered valid for the purpose of determining differences between the samples analyzed.

An analysis of the data presented in Table 3 indicates that with the possible exception of Sample C, vacuum distillation was effective in removing sufficient quantities of flavor-producing compounds, especially those related to staleness, to materially improve the flavor of the reconstituted products. Thus, the role of volatile carbonyls in the off-flavors of dry whole milk, as established in Part I of this study, is indicated again.

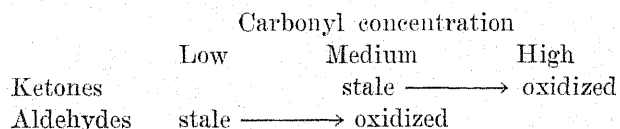
Data in Table 4 reveal that with the exception of the lower molecular weight aldehydes and ketones, the carbonyls identified are present as a result of heat

TABLE 4
Concentrations of carbonyls obtained from various dry whole milk products
(mg. per 10 lb.)

Carbonyl	Sample				
	A	B	C	D ^a	Control
Acetone	2.01	5.63	2.34	1.66	24.71
Butanone-2	0.07	0.24	0.31	0.35	3.04
Pentanone-2	0.06	0.35	0.61	0.39
Hexanone-2	0.30	1.20	0.97	0.17	0.23
Heptanone-2	0.27	0.44	0.96	0.02
Nonanone-2	0.04	0.09	0.18
Undecanone-2	0.02	0.03	0.06
Tridecanone-2	0.01	0.01	0.02
Pentadecanone-2	0.01	0.01
Formaldehyde	0.15	0.33	0.28	0.25	0.08
Acetaldehyde	0.22	0.84	1.05	0.22	0.08
Propanal	0.14	0.09	0.34
Pentanal	0.54
Hexanal	0.01	2.26	0.54
Heptanal	0.94	0.28
Octanal	0.35	0.09
Nonanal	0.25	0.02
Decanal	0.02	0.16
Dodecanal
2-Pentenal	0.02
2-Hexenal	0.03
2-Heptenal	0.29
2-Octenal	0.02
2-Nonenal	0.12
2-Decenal	0.06
2-Undecenal	0.03

^a Sample contained two mono- and two diunsaturated carbonyls of unknown identities.

processing and storage of the dry whole milks. The exact role of the different carbonyl classes in the various off-flavors is not clear. The dominant carbonyls in Samples B and D were saturated and unsaturated aldehydes, whereas Sample C contained only methyl ketones with the exception of formaldehyde and acetaldehyde, normal components of all products investigated. All three products were criticized as containing both oxidized and stale flavors. If the panel members were of one mind as to the nature of staleness, this suggests that the defect may be caused by more than one combination of compounds and that the off-flavor may change qualitatively with change in the concentrations of the responsible compounds. Although this study has not yielded entirely adequate data on the stale-oxidized relationship, the following scheme seems to account rather well for the information in Table 3.



This scheme assumes that ketone formation somewhat precedes aldehyde formation, that in general aldehydes exhibit lower flavor thresholds than ketones, and that both classes of compounds can impart the impression of staleness near threshold. The need for threshold and flavor characterization studies on indi-

vidual as well as mixtures of carbonyls is quite evident before definite conclusions concerning their significance in staling can be drawn.

Deodorization of milk fat, prior to the manufacture of dry whole milk, is apparently an effective means of improving the final flavor of dry whole milk. Methyl ketones are noticeably absent in products prepared from deodorized milk fat. The formation and removal of odd-numbered methyl ketones during steam-stripping of butterfat has been reported (12). In addition to flavor improvement due to the removal of ketones, the deodorization process forms and removes lactones (13) which are responsible for the coconut-like flavor of dry whole milk (10). The identification of saturated aldehydes in the product prepared with deodorized milk fat indicates that although the product was originally nitrogen-packed, autoxidation apparently occurred in one or more of the five cans of sample used.

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A METHOD OF CONCENTRATING RIPENED CHEESE VOLATILES FOR GAS CHROMATOGRAPHY

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SUMMARY

A method has been developed for concentrating the volatile organic compounds in ripened cheese for gas chromatographic analysis. The method consists of a rapid, room temperature churning of a cheese slurry and centrifugation of the resulting heated butter. The dry aromatic oil which is obtained is subjected to high vacuum distillation and the distillates are fractionated. With fully mature Blue Cheese, an organic layer separates from the distillate and is easily isolated and dried. With Cheddar Cheese and immature Blue Cheese, the distillates are extracted with ether and the extract evaporated in the presence of a small amount of high-boiling solvent, benzyl alcohol, which acts as a carrier for the volatiles to be analyzed.

Gas chromatography is a helpful supplement to the analytical techniques commonly used in cheese ripening and flavor research. One difficulty in its use is that of obtaining a sample suitable for injection into the apparatus. A suitable sample, as defined here, is that obtained from the original product by a relatively nondestructive process which will neither alter the volatile compounds already present nor create new ones. This volatile organic fraction should be free from excess water and organic solvents which mask the chromatogram and should contain enough volatiles for several gas chromatographic separations.

Earlier, an atmospheric steam distillation of aqueous Cheddar and Blue Cheese slurries from 8 and 36 lb. of cheese, respectively, followed by ether extraction of the distillate was reported by Jackson *et al.* (4, 5). This method produces the best yield of volatile components but steam distillation, especially at atmospheric pressure, carries with it the danger of altering some of the existing volatile compounds and causing further hydrolysis of protein and fat. Patton (7) has shown that steam vacuum distillation of butter oil, a relatively mild treatment, can produce methyl ketones. Also, making an aqueous slurry from cheese usually involves the addition of an equal part of water. From 8 lb. of cheese, seven liters of slurry are produced. The distillation of this volume of material involves either a very large-scale distillation or a time-consuming series of smaller distillations.

The aroma of ripened cheese apparently predominates in the fat phase (4). With this in mind, an analytical technique was developed here which included among its steps a separation of oil from cheese, vacuum distillation of the oil, fractionation of the distillate, and gas chromatographic analysis of the volatile organic fraction. An explanation of this method is the essence of the present report.

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ANALYTICAL PROCEDURES AND RESULTS

Churning and separation of fat. One thousand grams of Blue or Cheddar Cheese is cut into small cubes and blenderized with an equal weight of water. The slurry, at approximately 20° C., is agitated by hand or mechanical stirrer and enough 2 *N* HCl is added slowly to lower the pH to 4.2. Soon butter granules rise to the top. After agitation is terminated the granules clump together. This churned slurry is cooled at 5° C. for 30 min.

The resulting butter is heated rapidly in a water bath to 48° C. and the melted oil is centrifuged in an International Size 2 centrifuge at 2,000 r.p.m. for 5 min. Three layers, oil, water, and protein, separate in the cups. The aqueous layer is removed with a pipette and the oil, containing small amounts of protein and moisture, is poured from the solid protein residue into new centrifuge cups containing 15 to 30 g. of anhydrous magnesium sulfate. After a 5-min. centrifugation, a clear, dry oil with cheese aroma results and is poured into a distillation flask.

Distillation. The distillation apparatus consists of a one-liter round-bottomed flask connected directly to a vacuum trap which is connected to a T-shaped tube, one leg of which is attached to a McLeod gauge, the other to the vacuum pump (Figure 1). All connections and fittings are ground glass. After the vacuum pump is started, the contents of the distillation flask, agitated by a magnetic stirrer, are heated by an electrical heating mantle to 80° C. The vacuum trap is submersed in liquid nitrogen. When the pressure of the system is down to less than 5 μ of mercury, the stopcock leading to the vacuum pump is closed and the distillation of the closed system proceeds for 4–7 hr. In this time, the pressure increases to 25–50 μ (mercury). The vacuum is then released and the contents of the trap collected. For a test compound, ethyl butyrate (B.P. 121° C.), the yield of the distillation is approximately 30%.

Treatment of distillates and gas chromatographic analysis. Blue Cheese. The distillates obtained by the above procedure from fully ripened commercial Blue Cheese have a total volume of approximately 0.2–0.7 ml. and have separated into

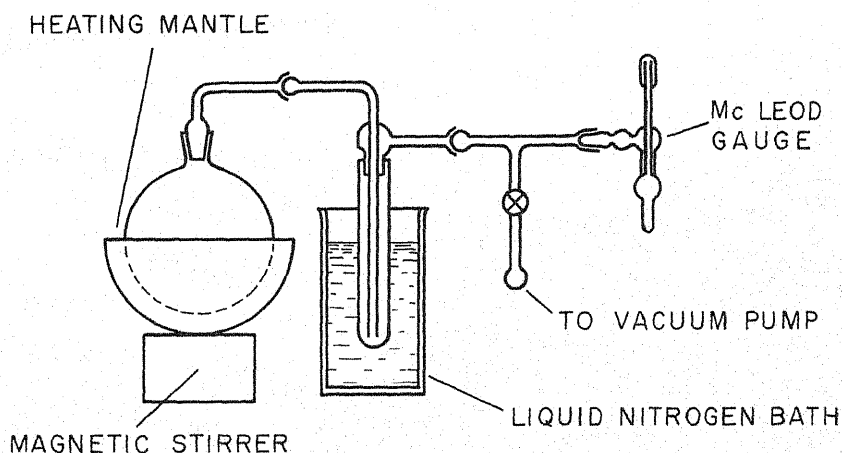


Fig. 1. Vacuum distillation apparatus.

two liquid layers. The aqueous phase (bottom) is removed with a syringe and a small amount of anhydrous magnesium sulfate is added to the remaining upper layer (organic). After standing overnight, the liquid is removed with a syringe from the settled drying agent. In this manner, a sample of pure organic volatiles (0.1–0.2 ml.) with an intense Blue Cheese aroma is obtained. A gas chromatogram of 18 μ l. of a typical sample at the given conditions is presented (Figure 2). Peaks, shown here, represent only a portion of the total number of compounds. Under these conditions, 2-nonanone and other higher boiling components have retention times longer than 1 hr.

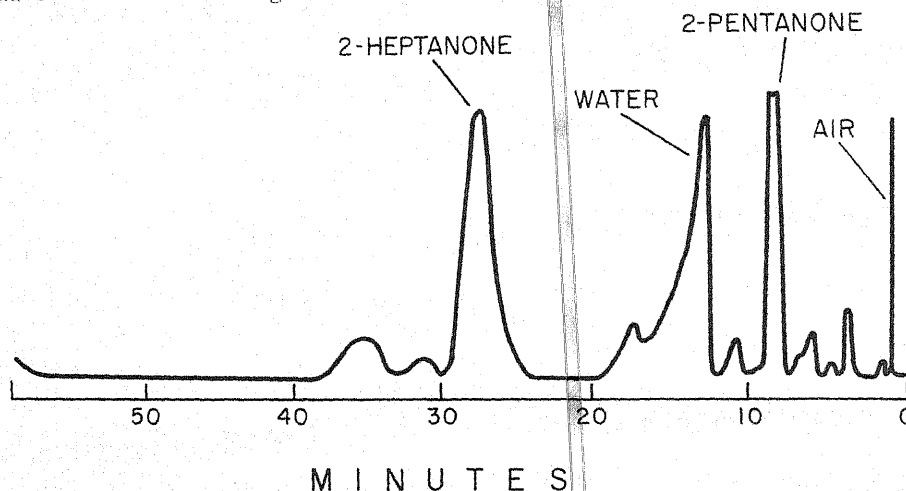


Fig. 2. Gas chromatogram of Blue Cheese volatiles.² Column temperature 80° C., 2 m.v. recorder span, flow rate 66 ml. per minute, column Carbowax 1540, filament current 270 m.a.

Distillates from underdeveloped or immature Blue Cheese fail to separate into layers and further fractionation by a technique described in the following section on Cheddar Cheese is necessary. Immature Blue Cheeses have a relatively hard body, a small amount of blue veins, and little flavor.

Cheddar Cheese. The distillates from fully ripened Cheddar Cheese (0.1–0.5 ml.) and those of underdeveloped Blue Cheese do not separate into layers even when saturated with potassium carbonate. To eliminate the excess water, which obscures the presence of certain compounds, the distillate is extracted with 3 ml. of ether in 1-ml. portions and the extract is dried with anhydrous magnesium sulfate. To the dried extract is added 50 μ l. of benzyl alcohol (B.P. 205° C.), and the ether is evaporated from the mixture at 35° C. until the ether aroma is no longer detectable in the liquid residue. If the benzyl alcohol is not added to the ether extract, a large percentage of the volatile compounds will be lost in the evaporation to near dryness, because of their relatively low boiling points and trace concentrations.

² Compounds indicated in this and other figures have been identified tentatively by retention time and functional group test. Only acetic and butyric acid in Figure 4 have been identified positively by infrared spectrophotometry.

The benzyl alcohol retains the desired compounds and is a suitable carrier for injection into the gas chromatography apparatus. It does not interfere with the chromatogram because of its high boiling point and it will remain in the column until the temperature is raised far above the temperature of analysis. Gas chromatographic examination of 25 μ l. of pure benzyl alcohol shows no impurities.

A typical gas chromatogram of the lower boiling point components in a 25- μ l. sample obtained by the above technique from the distillate of an aged Cheddar Cheese is shown (Figure 3).

For the analysis of some compounds in the aged Cheddar Cheese distillate the final ether extraction step is not necessary. If 100 μ l. of the distillate is directly injected into the gas chromatography apparatus fitted with a Carbowax 1540 column at 60° C. and a helium flow rate of 162 ml/min, a satisfactory chromatogram of polar compounds with boiling points of less than 100° C. (approx.) and nonpolar compounds with boiling points less than 130° C. is possible (Figure 4). The peaks of these compounds will appear before the water

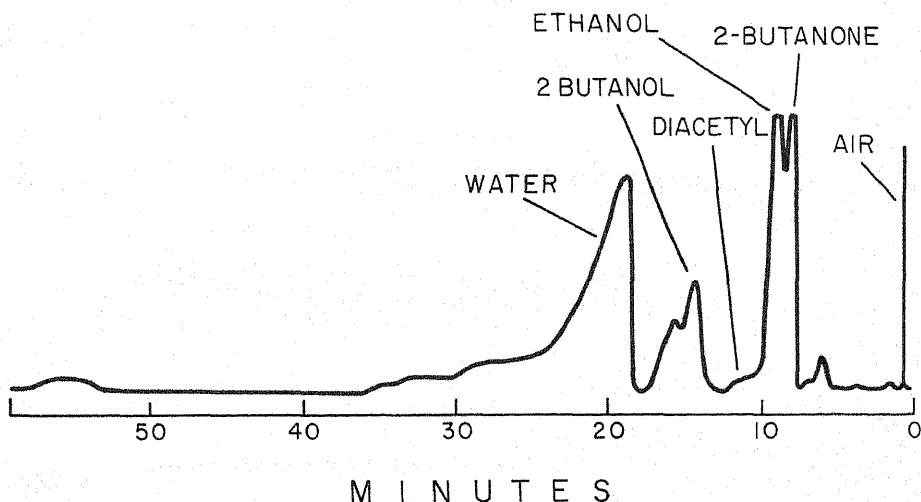


FIG. 3. Gas chromatogram of Cheddar Cheese volatiles in benzyl alcohol carrier. Column temperature 67° C., 2 m.v. recorder span, flow rate 50 ml. per minute, column Carbowax 1540, filament current 278 m.a.

peak, which is delayed on the polar column. Also, by raising the temperature of the column to 150° C. upon appearance of the water peak, the water will be quickly eluted and the high boiling compounds will follow. Decreasing the recorder sensitivity to 10 mv. minimizes the base line change, due to the temperature increase.³

³ The gas chromatography apparatus used in this research was an Aerograph Model A-100 equipped with a Varian recorder with a 1-10 mv. range selector switch. With other commercial apparatuses having thermal conductivity detectors, the described technique may result in a much greater base line change.

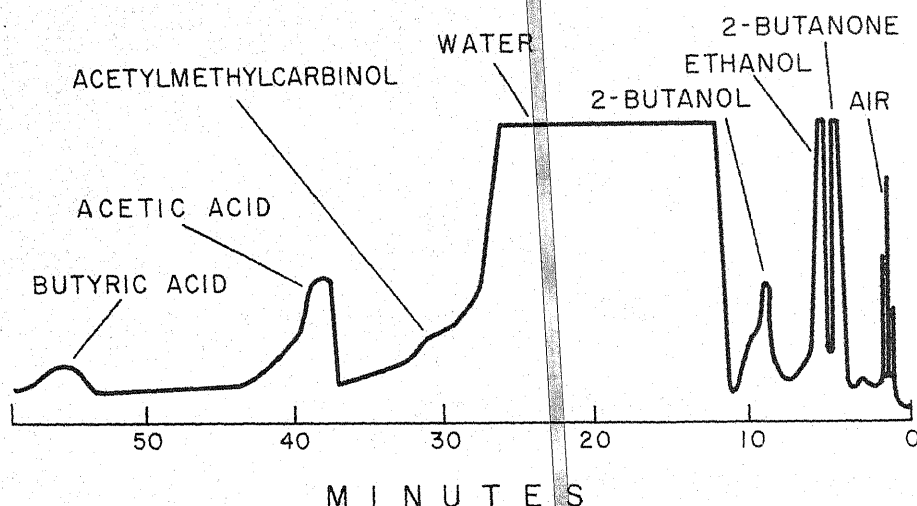


Fig. 4. Gas chromatogram of distillate from Cheddar Cheese oil. Column temperature 60° C. until water peak, recorder span 2 m.v. before water peak, 10 m.v. after water peak, flow rate 162 ml. per minute, column Carbowax 1540, filament current 273 m.a.

DISCUSSION

By removing the oil from cheese, a quick separation of a good portion of the volatiles from the easily decomposed protein and the bulk of the water is accomplished. Previous workers have separated the oil from cheese by a variety of methods which include centrifuging cheese slurries at 90° C. (1), pressing cheese-sand mixtures in a hydraulic press (6), and churning cheese-water mixtures by mechanical agitation at 40° C. for 3 hr., followed by ether extraction (3). The present churning technique has the advantages of being very rapid and accomplished at below room temperatures. Heat treatment is kept to a minimum in the separation of oil from the resulting butter which oils off easily. The yield of oil, 60%, is far greater than the yields obtained by using a hydraulic press or centrifuging cheese slurries in this laboratory.

The churning rate depends upon pH and temperature. Agitation of the slurry at pH 4.0–4.2 and approximately 20° C. results in almost instantaneous churning. Lower temperatures and higher pH's prolong the process considerably. This optimum pH range is the same as that in which the churning of cream occurs most rapidly (2).

The pH adjustment serves another purpose. At the normal pH of ripened Blue and Cheddar Cheese, approximately 6.0 and 5.4, respectively, the fatty acids are predominantly in the salt form, whereas at pH 4.2 they are predominantly in the acid form and can partition between the water and fat phase.

High-vacuum distillation is a fairly efficient method of stripping the desired volatiles from the tenacious oil at moderate temperatures. Since only the cheese oil is distilled, it is possible to keep the distillation on a small scale, even when starting with several pounds of cheese.

At no time in the entire procedure are quantities of solvents employed which might cause artifacts due to the presence of impurities.

The yields of volatile components, by the described techniques, vary with the variety of cheese used and the degree of ripening of the cheese. Fully ripened Blue Cheese gives enough pure volatile essence from 2.2 lb. of cheese for detailed analysis on several types of column under varying conditions. Ripened Cheddar gives appreciably less, but still enough for two or three separations.

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ENZYMIC DEGRADATION OF β -CASEIN BY A SNAKE VENOM PREPARATION

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SUMMARY

The action of a snake venom preparation from *Crotalus adamanteus* on β -casein has been studied. The action resulted in the formation of a turbidity, which was measured with time in the spectrophotometer. The effects of temperature, metal ions, pH, and ionic nature of the medium have been observed. The reaction appears to be proteolytic in nature, being accompanied by an increase in ninhydrin-positive material and resulting in the formation of at least four different fractions from β -casein. The proteolytic activity always preceded the precipitate formation. The material precipitated during the reaction was essentially phosphorus-free and comprised 20-30% of the original β -casein.

During the course of a study on the nature of the phosphate linkages in casein fractions, it had been noted that when β -casein is incubated with a snake venom preparation having phosphodiesterase activity, turbidities developed (9). This phenomenon is accompanied by a shift in pH toward the acid side, as had been previously noted by Perlmann (14), who took this to mean the cleavage of phosphodiester bonds. However, this conclusion has been recently challenged (7, 9). The present communication reports some of the properties and characteristics of the reaction of β -casein with an enzyme preparation obtained from *Crotalus adamanteus*.

MATERIALS AND METHODS

β -Casein. The casein was prepared from whole casein by fractionation in aqueous urea solutions (6). The preparation used in these studies had a moisture content of 7.62% and a total phosphorus content of 0.61% on a moisture-free basis.

Enzyme preparation. The preparation used was obtained as a dry powder from Ross Allen's Reptile Institute, Silver Springs, Florida, and was fractionated by the method of Sinsheimer and Koerner (16). The precipitate formed with 0-40% acetone was found to be most active in causing turbidity with β -casein. This fraction, obtained in the first acetone cycle, was dissolved in water and used as the enzyme preparation. It also had phosphodiesterase activity. It contained 8 mg. protein/milliliter as determined by the micro-Kjeldahl procedure, using the factor 6.25. The enzyme solution retains full activity when stored at 4° C. for 3 mo.

Measurement of turbidity. The turbidity measurements were made in the Beckman Spectrophotometer (Model DU) at a wave length of 600 m μ . The reaction was carried out in cuvettes of 1-cm. path length at a constant temperature maintained by means of thermospacers.

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Measurement of proteolysis and pH. The proteolysis was measured by the increase of ninhydrin-positive material of the entire reaction mixture as described previously (9). The modified ninhydrin method of Moore and Stein (12) was employed, and the results were quantitated by comparison with a standard leucine curve. It is necessary to keep in mind that the quantitative data are taken only as an approximation of the degree of proteolysis, for reasons previously stated (9). The pH determinations were made at room temperature before and after the reaction, using the glass electrode.

Measurement of nitrogen and phosphorus. Total nitrogen was determined by a modification of the micro-Kjeldahl method of Ma and Zuazaga (10), in which a mixture of K_2SO_4 and HgO was used as catalyst during the digestion procedure. Inorganic orthophosphate was determined on trichloroacetic acid filtrates (final concentration—10% TCA) of protein solutions by the method of Fiske and Subbarow (5). Total phosphorus was determined as above after prior digestion with H_2SO_4 (17).

EXPERIMENTAL PROCEDURE

The following types of experiments were carried out to study the properties and characteristics of the action on β -casein of the snake venom fraction noted above.

Turbidimetric studies. In these studies, the desired amount of enzyme was placed in the cuvette, which was then allowed to come to temperature, usually $38^\circ C.$, unless otherwise indicated, in the spectrophotometer. The remaining solution, including the casein and any other substances pertinent to the experiment, was placed in a tube immersed in a water bath at $38^\circ C.$ At zero time, the contents of the tube was added to the cuvette to bring the final volume to 3.0 ml. The pH of the solution in the tube had been previously adjusted, and since the volume of the enzyme solution was, in general, less than 0.2 ml., there was very little change in pH. This was verified by independent mixing experiments. The first reading of the optical density was made 1 min. after mixing, and readings were continued at intervals until 30 min. had elapsed, as indicated in the accompanying figures. The pH was then measured, and a plot of the increase of optical density at $600 m\mu$ with time was made. The exact composition of the reaction media is given below, under Results.

Proteolytic studies. These studies were carried out separately from the turbidimetric studies. Tubes were incubated in a water bath at $37^\circ C.$; 0.1-ml. aliquots of the reaction mixture were taken at zero time and every 3 min. thereafter until the fifteenth minute, when aliquots were taken every 5 min. until the experiment was concluded after 30 min. The increase of ninhydrin positive material was measured as previously described (9) and compared with the turbidimetric data. Data are reported in Table 1 in terms of bonds split/30,000 mol. wt.

Distribution of nitrogen and phosphorus. To determine the distribution of N and P between the supernatant and precipitate, the following experiment was performed. Two reaction tubes containing 75 mg. of β -casein, 0.75 mg. of

TABLE 1
Extent of proteolysis during the action of snake venom on β -casein

System	Bonds hydrolyzed ^a	
	(30,000 mol. wt.)	
	After 6 min.	At end 30 min.
Control ^b	1.2	3.7
Control (T = 26° C.)	1.0	3.2
-Mg ⁺⁺	1.3	2.4
Substrate = 400		
Enzyme I	0.7	2.1
+ 3.3 × 10 ⁻³ M versene (-Mg ⁺⁺)	0	0
+ 3.3 × 10 ⁻³ M versene (-Mg ⁺⁺)	0	0
+ 8.2 × 10 ⁻³ M veronal	0.8	2.5
+ 3.3 × 10 ⁻¹ M veronal	0	0
+ 3.3 × 10 ⁻¹ M NaCl	0.7	2.7
+ 6.6 × 10 ⁻¹ M NaCl	1.2	2.7

^a All values reported are the averages of at least two independent determinations.

^b Control system consisted of a substrate-to-enzyme ratio of $\frac{100}{1}$ in an unbuffered medium of pH 9.5 at 36° C. containing 3.3 × 10⁻³ M MgCl₂. All other systems were the same except as indicated.

enzyme, and 50 μ moles of Mg⁺⁺ in a volume of 10 ml., pH 9.7, were incubated at 36° C. A third control tube contained no enzyme. Aliquots were taken at 0, 15, and 30 min. to determine total N, total P, and inorganic P of the supernatant after the solution had been clarified by centrifugation at room temperature at 2,000 g for 30 min. The extent of proteolysis was also measured, using the whole reaction mixture as described above, and the pH was also determined at the end of 30 min. The precipitate could be further fractionated, as will be described under Results and Discussion.

RESULTS

Turbidimetric studies. Figure 1 is the plot of a typical experiment in which snake venom was incubated at 38° C. in a final volume of 3.0 ml. with β -casein (0.5%) (substrate to enzyme weight ratio of 100:1). The reaction underwent a pH shift of 1.2 units toward the acid side from an initial pH of 9.5, after correcting for pH changes in the control. Also present in the medium was MgCl₂ (3.3 × 10⁻³ M). It is noted that an S-shaped curve was obtained with a lag of about 4 min. and with a slope of $\frac{0.243 \text{ O.D. units}}{\text{minutes}}$ for the straight line portion of the curve (between 4 and 11 min.). When the enzyme preparation was boiled for 5 min. and then incubated with the casein under the same conditions, there was no reaction. The addition of boiled enzyme to the system described above was neither inhibitory nor stimulatory. When the substrate-to-enzyme ratio was varied over an eightfold range by varying the enzyme concentration, the series of plots illustrated in Figure 2 was obtained. As the β -casein to snake venom ratio increased, the lag period increased, and the slope of the straight-line portion of the curve decreased, as did the pH shift. Also shown in Figure 2 is the plot obtained when the temperature of the reaction was reduced to 25° C. with a substrate-to-enzyme ratio of 100:1. The lag

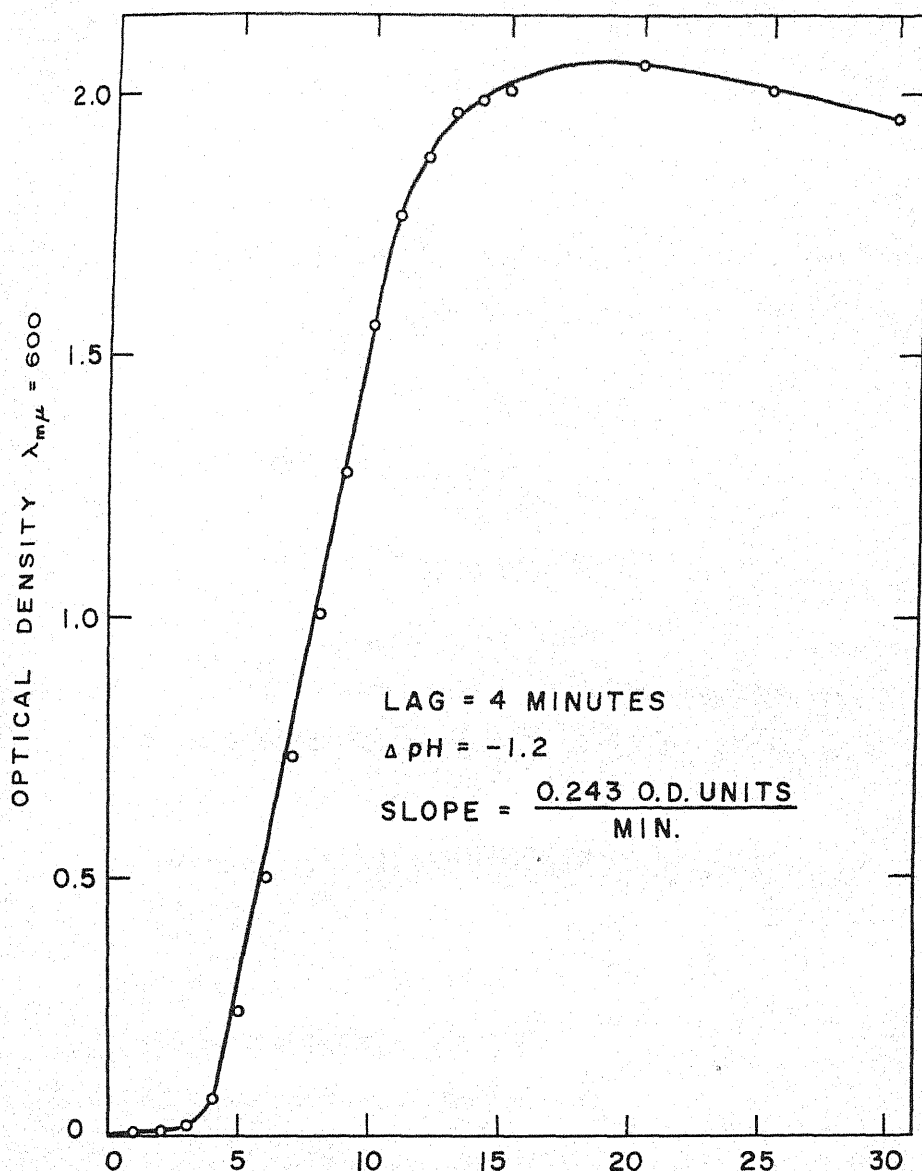


FIG. 1. Incubation of β -casein with snake venom at 38° C. in the presence of 3.3×10^{-3} M $MgCl_2$ at an initial pH of 9.5.

period was greatly extended, and the slope was reduced by approximately one-half.

It was found that when calcium ions replaced magnesium, the reaction proceeded at the same rate as in the presence of magnesium. However, the omission of divalent metal ions resulted in an increase in the lag and decrease in the slope, as seen in Figure 3. The addition of boiled enzyme to the system

	365
of mold frag-	
try. BURDET	369
Inction and on	377
H. O. HENDER-	
	385
	397
	A75
	413
k powder in ice	
milking and udder	419
	433
SPIELMAN, W. E.	
bin upon milk and	441
	449
ION	459
W. J. TYLER AND	
brane." LEROY S.	463
	471
milk. KENNETH J.	
FRANK CONE	483
time heater by pre-	
SANDERS	499
	A91
	505
ation administered to	
I. DUNCAN AND C. F.	539
	545
rows over a three-week	
plement to the normal	551
SALISBURY	
sh water during manu-	563
lasses or cane molasses	
n. R. C. MILLS, C. C.	571
ration composition. C.	579
HART	
H, S. M. HAUGE, AND J.	585
	A105

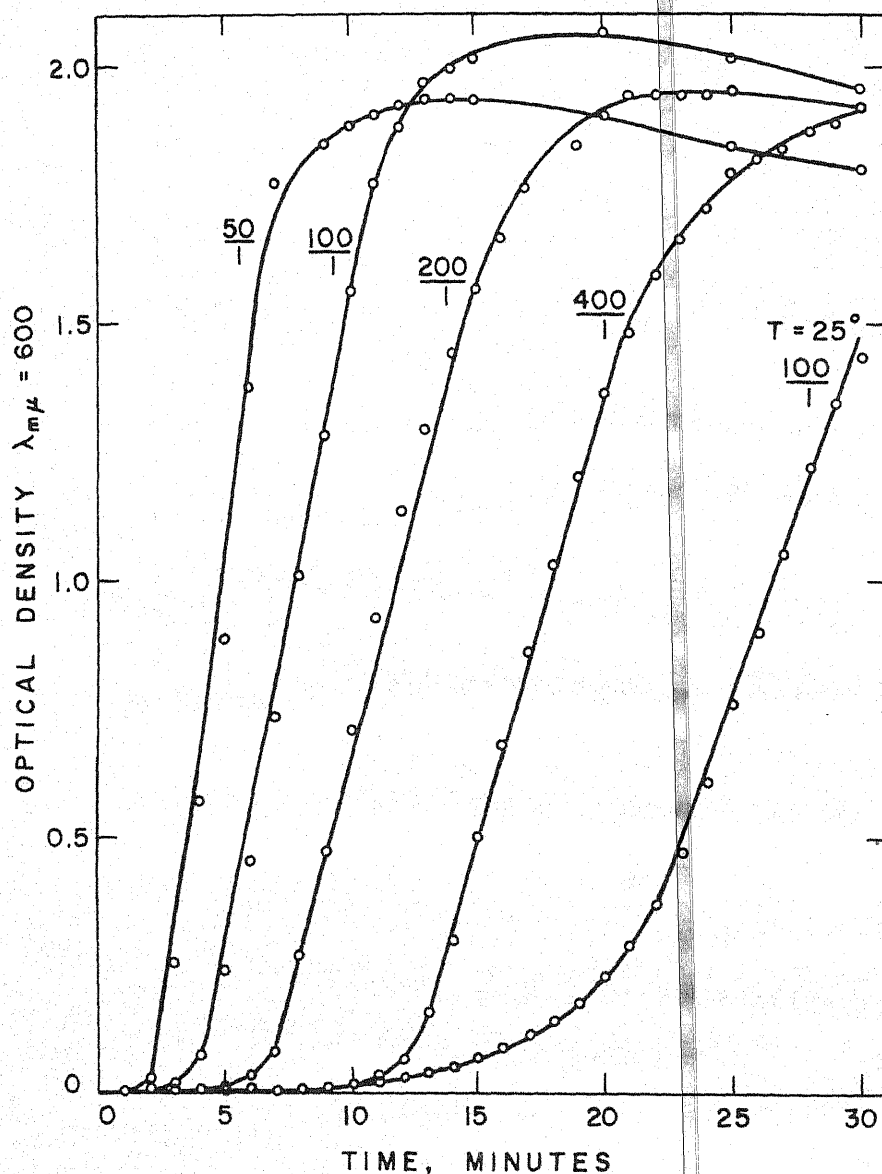


Fig. 2. Incubation of β -casein with snake venom at 38°C .—varying substrate to enzyme concentration in the presence of $3.3 \times 10^{-3} M$ MgCl_2 at an initial pH of 9.5. $(\frac{50}{1})$ slope = $\frac{0.348 \text{ O.D. units}}{\text{minutes}}$, $\Delta\text{pH} = -1.4$, $(\frac{100}{1})$ slope = $\frac{0.243 \text{ O.D. units}}{\text{minutes}}$, $\Delta\text{pH} = -1.2$, $(\frac{200}{1})$ slope = $\frac{0.185 \text{ O.D. units}}{\text{minutes}}$, $\Delta\text{pH} = -1.0$, $(\frac{400}{1})$ slope = $\frac{0.166 \text{ O.D. units}}{\text{minutes}}$, $\Delta\text{pH} = -0.6$. $T = 25^\circ\text{C}$. $(\frac{1000}{1})$ slope = $\frac{0.133 \text{ O.D. units}}{\text{minutes}}$, $\Delta\text{pH} = -1.1$.

CONTENTS

v

<i>Peppergrass silage.</i> AVERY D. PRATT	365
<i>A new quantitative method for estimation of total combined length of mold fragments in butter.</i> P. R. ELLIKER	369
<i>The determination of citric acid in milk products by cerate oxidimetry.</i> BURDET HEINEMANN	377
<i>The effect of iodinated casein (protamone) on milk and butterfat production and on the ascorbic acid content of the milk.</i> A. H. VAN LANDINGHAM, H. O. HENDERSON AND CHARLES E. WEAKLEY, JR.	385
<i>Program thirty-ninth annual meeting</i>	397
<i>Abstracts of literature</i>	A75

JUNE, NO. 6

<i>Red mold on blue cheese.</i> B. W. HAMMER AND JOSEPH C. GILMAN	413
<i>Observations on the use of roller process sweet cream buttermilk powder in ice cream.</i> E. L. THOMAS AND W. B. COMBS	419
<i>The action of the mechanical milker in relation to completeness of milking and udder injury.</i> W. E. PETERSEN	433
<i>The effect of thyroidectomy on lactation in the bovine.</i> A. A. SPIELMAN, W. E. PETERSEN AND J. B. FITCH	441
<i>The effect of complete evacuation of the mammary gland by pitocin upon milk and fat production.</i> C. B. KNOTT AND W. E. PETERSEN	449
<i>Changes in bacterial counts of stored ice cream mix.</i> F. E. NELSON	459
<i>A simplified method of estimating 305-day lactation production.</i> W. J. TYLER AND A. B. CHAPMAN	463
<i>The structure and properties of the natural fat globule "membrane."</i> LEROY S. PALMER	471
<i>Improving the quality of Swiss cheese by clarification of the milk.</i> KENNETH J. MATHESON, GEORGE P. SANDERS, LLOYD A. BURKEY AND J. FRANK CONE	483
<i>Prevention of milkstone formation in a high-temperature-short-time heater by pre-heating milk, skim milk and whey.</i> R. W. BELL AND C. F. SANDERS	499
<i>Abstracts of literature</i>	A91

JULY, NO. 7

<i>Lactose and its utilization: A review.</i> EARLE O. WHITTIER	505
<i>Observations on the effects of an anterior pituitary preparation administered to lactating dairy cows.</i> J. F. SYKES, I. A. GOULD, C. W. DUNCAN AND C. F. HUFFMAN	539
<i>The influence of a synthetic thyroprotein when fed to dairy cows over a three-week period.</i> RALPH P. REECE	545
<i>A controlled experiment in feeding wheat germ oil as a supplement to the normal ration of bulls used for artificial insemination.</i> G. W. SALISBURY	551
<i>Retention of mold fragments by butter, buttermilk and wash water during manufacture of butter.</i> P. R. ELLIKER	563
<i>Utilization of urea and growth of heifer calves with corn molasses or cane molasses as the only readily available carbohydrate in the ration.</i> R. C. MILLS, C. C. LARDINOIS, I. W. RUPEL, AND E. B. HART	571
<i>Rumen synthesis of the vitamin B complex as influenced by ration composition.</i> C. C. LARDINOIS, R. C. MILLS, C. A. ELVEHJEM, AND E. B. HART	579
<i>Carotene losses in freshly cut plant tissues.</i> R. K. WAUGH, S. M. HAUGE, AND J. H. HILTON	585
<i>Abstracts of literature</i>	A105

AUGUST, NO. 8

<i>Papers presented at the annual meeting</i>	591
<i>The training and employment of milk sanitarians: A committee report.</i> H. F. JUDKINS, F. H. HERZER, L. H. BURGWARD, P. H. TRACY, C. E. WYLIE, AND C. L. ROADHOUSE	691
<i>The 39th annual meeting of the American Dairy Science Association.</i> R. B. STOLTZ	701

SEPTEMBER, NO. 9

<i>A breed comparison in the vitamin D content of milk with notes on a modified technique for the vitamin D assay of low-potency fats and oils.</i> G. C. WALLIS	733
<i>Lactic acid in dairy products. I. Application of the Hillig method.</i> I. A. GOULD	743
<i>Lactic acid in dairy products. II. Relation to flavor, acidity measurements, bacterial count and methylene blue reduction.</i> I. A. GOULD AND J. M. JENSEN	753
<i>A technique for the simultaneous measurement of semen quality and testis histology in vitamin A studies of the dairy bull.</i> R. E. ERE, F. N. ANDREWS, J. F. BULLARD, AND J. H. HILTON	769
<i>Seasonal and geographical variations in the citric acid content of defatted milk solids.</i> BURDET HEINEMANN	773
<i>Further studies on bloat.</i> S. W. MEAD, H. H. COLE, AND W. M. REGAN	779
<i>Non-permeability of the mammary gland to thyroid hormone.</i> E. P. REINEKE AND C. W. TURNER	793
<i>Abstracts of literature</i>	A119

OCTOBER, NO. 10

<i>The effect of the method of manufacture of butteroil on its keeping quality.</i> M. S. EL-RAFFEY, G. A. RICHARDSON, AND J. L. HENDERSON	807
<i>The nutritive value of alfalfa hay. II. Starch and glucose as supplements to an all alfalfa hay ration.</i> C. F. HUFFMAN AND C. W. DUNCAN	821
<i>A standard for evaluation of dairy sires proved in dairy herd improvement associations.</i> N. N. ALLEN	835
<i>The ratio of ascorbic, nicotinic, and pantothenic acids, riboflavin and thiamin in late summer milk.</i> ARTHUR D. HOLMES, CARLETON P. JONES, ANNE W. WERTZ, KATHERINE ESSELEN, AND BEULA V. McKEY	849
<i>A note regarding the speeds of Babcock centrifuges.</i> B. L. HERRINGTON	857
<i>The value of kelp meal in rations for dairy cattle.</i> M. H. BERRY AND K. L. TURK	861
<i>Effect of colostrum on the vitamin A and carotene content of blood plasma of newborn calves.</i> L. A. MOORE AND M. H. BERRY	867
<i>The effect of storage on the retention of certain members of the vitamin B complex.</i> C. C. LARDINOIS, C. A. ELVEHJEM, AND E. B. HART	875
<i>Testing for extraneous matter in cheese.</i> RAYMOND MIERSCH AND WALTER V. PRICE	881
<i>Abstracts of literature</i>	A147

NOVEMBER, NO. 11

<i>The determination of tannic substances in commercial cocoa powders.</i> W. S. MUELLER AND J. W. KUZMESKI	897
<i>The recognition of the influence of age on butterfat percentage when calculating mature equivalents.</i> D. N. PUTNAM, G. A. BOWLING, AND C. T. CONKLIN	903
<i>Temperature-time relationships for high-temperature-short-time sterilization of evaporated milk.</i> H. R. CURRAN, R. W. BELL, AND F. R. EVANS	909
<i>Effects of temperature and time of sterilization upon properties of evaporated milk.</i> R. W. BELL, H. R. CURRAN, AND F. R. EVANS	913

CONTENTS

vii

<i>A new method for indexing dairy bulls.</i> V. A. RICE	921
<i>The optimum emphasis on dams' records when proving dairy sires.</i> JAY L. LUSH	937
<i>The relative concentrations of dam and fetal liver glycogen.</i> C. B. KNOTT AND W. E. PETERSEN	953
<i>The stimulation and inhibition of milk secretion in goats with diethylstilbestrol.</i> JOHN P. MIXNER, JOSEPH MEITES, AND C. W. TURNER	957
<i>The control of diarrhea ("white scours") of new-born dairy calves by means of serum and sulfaguanidine.</i> G. H. WISE AND G. W. ANDERSON	965
<i>Abstracts of literature</i>	A159

DECEMBER, NO. 12

<i>Relation of various factors to the breeding efficiency of dairy animals and to the sex ratio of the offspring.</i> R. A. HILDER, M. H. FOHRMAN, AND R. R. GRAVES	981
<i>Bacteriological evaluation of ice cream.</i> F. E. NELSON	993
<i>Sulfur compounds as disinfecting agents for dairy equipment.</i> W. S. MUELLER, EMMETT BENNETT, AND JAMES E. FULLER	1007
<i>Live-weight gains of pasture-fed dairy heifers.</i> W. B. NEVENS	1011
<i>The use of urea in commercial dairy feeds.</i> W. H. HASTINGS	1015
<i>Circulation of the Journal of Dairy Science</i>	1023
<i>Author index of original articles</i>	1057
<i>Subject index of original articles</i>	1065
<i>Abstracts of literature</i>	A177
<i>Author index of abstracts</i>	A187
<i>Subject index of abstracts</i>	A199
<i>Title page for year 1944</i>	i
<i>Editorial board</i>	ii
<i>Table of contents</i>	iii
<i>Errata</i>	viii

ERRATA

Vol. XXVI, No. 12, page A228

The first sentence in abstract 532 should read: "This investigation deals with the vacreator, a vacuum pasteurizer for cream for butter making, developed in New Zealand."

Vol. XXVII, No. 3, page A35

Line 11 in abstract 70 should read: "The phage infection usually occurs from the 'whey fog' from a whey separator. . . ."

Vol. XXVII, No. 4, page A70, line 15

Conclusion 6 should read: "It cannot be safely assumed: . . ."

Vol. XXVII, No. 8, page 673

The last line in the first paragraph should read: ". . . 34.0-49.0 μ g. protein and 0.57-0.86 μ g. lipide phosphorus were adsorbed per . . ."

Vol. XXVII, No. 10, pages 851, 853 and 855

Running headlines should read: "VITAMINS IN SUMMER MILK."

Vol. XXVII, No. 10, pages 850 and 854

Running headlines should read: "ARTHUR D. HOLMES ET AL."

Vol. XXVII, No. 10, page 812

Table 1, column 3: 0.151 should read 0.0151.

JOURNAL OF DAIRY SCIENCE

VOLUME XXVII

JANUARY, 1944

NUMBER 1

THE RÔLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS. I. MILK PROTEINS

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INTRODUCTION

The study of the foaming problem is of considerable interest in the dairy industry as well as several other industries. Some practical problems such as churning of cream, shrinkage of ice cream, whipping of cream or condensed milk, prevention of milk solids losses in manufacturing processes, foaming of casein in paper-coating, and the efficiency of pasteurization are directly related to the foam problem.

The surface-active constituents of milk contributing to its foaming properties, in all probability, are involved also in other physico-chemical problems such as creaming of milk, rebodding of cream, insolubility of milk powders and in the various activities at the fat globule interface.

A survey of the literature indicated the desirability of undertaking a systematic study of the fundamental foam problem in milk products. In the present paper, the apparatus used in these studies and the units of foam measurements are described, and the foaming properties of the major milk proteins are reported.

REVIEW OF LITERATURE

At the beginning of the present century, Siedel and Hesse (40) presented quantitative proof of a protein accumulation in the milk foam. This work, confirmed later by others (20, 38) marked the start of the present state of confusion as to what protein is responsible for the foaming of milk. Siedel (39) introduced the notion that the foaming substance in milk is other than its two major proteins—casein and albumin. Subsequent efforts were directed towards the finding of the foaming substance in the minor fractions of milk proteins.

Rahn (31) claimed that milk contains a special foam compound, which is probably the protein that surrounds the fat globules. This suggestion did

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not clarify the question to any extent. The protein of the stabilizing film on fat globules in milk has been identified as (a) albumin, (b) glycoprotein, (c) globulin-like protein, (d) new protein, (e) casein and (f) a mixture of proteins (6).

Hekma and Brower (15) concluded from microscopical studies that the separator slime came from collapsed foam cells. Grimmer and Schwartz (13) studied the nature of the foaming material in milk by analyzing the separator slime, and found that it contained only 36 per cent casein in the protein fraction. The other 64 per cent protein was claimed to be a new protein probably identical with the foaming substance of milk. Rahm and Sharp (32) called this substance "Schaumstoff."

Ansbacher, Flanigan and Supplee (1) eluted a protein fraction from high-foaming commercial casein. The eluted casein lost its foaming ability, whereas the elution product showed remarkable foaming. There was no definite conclusion as to the identity of this foaming substance, but a globulin with a sterol prosthetic group was suggested. According to the authors, the foaming compound can be separated from milk after removing all the major proteins of milk.

Sharp, Myers, and Guthrie (38) analyzed separated milk foam for the protein fractions. They concluded that there is no preferential accumulation of any major protein fraction in the foam. Sharp and Krukovsky (37) reported the isolation of an agglutinin from milk, which is responsible for the clustering of solid fat globules and the creaming of milk, and also for the foaming of skim milk separated at 50° C.

The modern conception of the factors and forces governing different stages in the existence of a foam will not be reviewed because they have recently been reviewed by Berkman and Egloff (3, 4).

The present investigation represents an attempt to establish a better understanding of the rôle of milk proteins involved in the foaming of milk and some of its products. It includes the isolation and study of the foaming properties of casein, lactalbumin and lactoglobulin.

EXPERIMENTAL

The foam apparatus. The foam measuring apparatus used in the present work is shown in figure 1. It is a modification of the type used by Hansley (14) and others (5, 8, 12, 18). The chief improvement is the provision for separating the foam from the liquid. A known volume of air is forced at a measured pressure through a sintered glass disc into the layer of liquid above it. The volume and the stability of foam which is formed are measured at the desired temperature.

Foam measurements. In standardizing the apparatus, the most reproducible results were obtained with separated milk under the following conditions: 1) volume of sample, 50 ml., 2) time of forcing air through the

sample, 20 seconds, 3) manometer pressure, 30 mm. of mercury and 4) reserve pressure on storage tank, 5 pounds per square inch (0.35 kg./cm.^2).

The volume of air that passes through the sample varies linearly with the temperature from 195 cc. at 5° C. to 270 cc. at 55° C. with a range of ± 2 per cent.

To determine the comparative quality of a foam, the sample was tempered in the water bath at the desired temperature and two measurements were obtained. 1) The foam height which is the reading taken immediately after shutting off the air pressure. 2) The average duration of half the

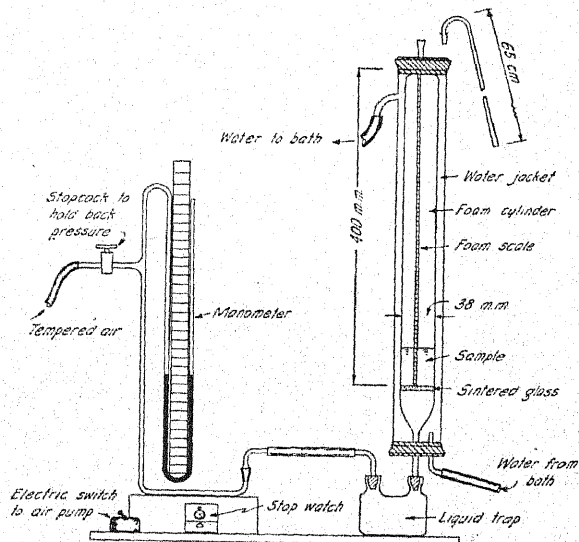


FIG. 1. Foam apparatus. The apparatus consists of a glass cylinder tapered at the lower end to form a ground glass joint. A sintered glass plate is sealed into the tube above the tapered portion. The upper end is sealed to a ground glass joint which permits a connection of a bent glass tube. The cylinder is surrounded by a removable water jacket connected to a pump that circulates the water from a thermostatically controlled water bath. The air pressure is generated by an air compressor provided with a storage tank, a gauge to indicate air storage pressure and a needle valve to control air volume and hence the working air pressure. The temperature and humidity of the air are controlled. A capillary tube mercury manometer connected to the air circuit indicates the pressure to the disc.

volume of the original foam produced, expressed in seconds. The latter is a measure of the foam stability and is termed "half-volume time."

The half-volume time: The introduction of the half-volume time unit proved to be of value in the present study where the stability of a static foam is to be compared with that of dynamic foam, and where the foams do not obey the same subsidence equation. The mathematical equations to calculate the foam life proposed by Lederer (18), Bikerman (5) and Ross and

Clark (8, 35) are not applicable to milk at all temperatures. Figure 2 shows the rate of collapse of raw separated milk foam (0.03 per cent fat) at different temperatures (the time factor in this figure is on a logarithmic scale). It is quite evident that no one formula can be used to calculate the average life of a bubble at all these temperatures. There is an apparent rest period for the separated milk foam before it starts to break down—after which the foam may either gradually subside or explode partially or completely. The "half-volume time"² was found to be the best measurement for comparing these foams. It has the advantage over reading the foam height after a certain time interval, which has been used in research on milk foams (19, 22, 36) in that it shows the relative stability of foam that sub-

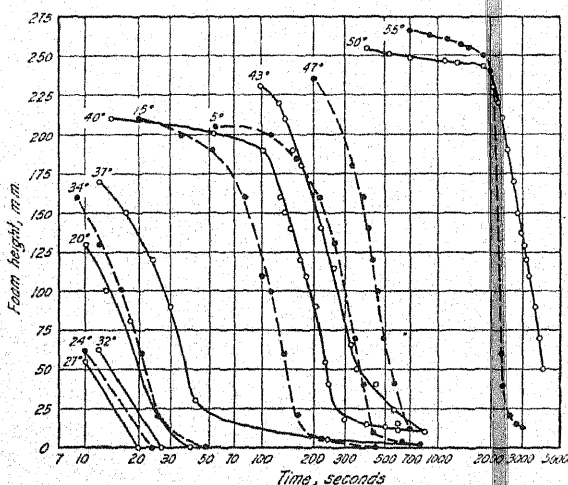


FIG. 2. The effect of temperature on the rate of collapse of raw skim (0.03 per cent fat) foam.

sides completely before the time interval or remains unchanged after the chosen time. This measurement, however, has the disadvantage of being time-consuming and requires constant observation of the foam until it breaks to half its volume. In order to minimize this disadvantage and still get comparable results, an upper limit for the stability was set. This limit was selected as 3,000 seconds, which is approximately the highest value obtained for milk and separated milk foams within the limits of temperature (5 to 55° C.) chosen in this study. The half-volume time of the samples studied at different temperatures seems to be independent of the foam height. This unit can be readily converted to Σ , the average life of a foam bubble, in case of logarithmic subsidence, using the equation $\Sigma = \frac{\text{half-volume time}}{2.303 \log 2}$.

² After completion of this work, a somewhat similar unit of measuring the foam stability was used by F. Schultz (Trans. Faraday Soc. 38: 85-93, 1942) which is called "foam-time." An excellent account of this unit of foaminess is presented in his paper.

Other measurements. The surface tension measurements were made by a Cenco-du-Noüy interfacial tensiometer. The readings were made within one minute after the surface had been renewed by stirring. Viscosity determinations were made by the Ostwald viscosimeter. For the determination of nitrogen a semi-micro Kjeldahl distillation apparatus, described by Redemann (33) was used, and 30 per cent hydrogen peroxide was added to aid the final oxidation. The pH values were checked with a quinhydrone electrode using a saturated calomel cell and a gold electrode.

ISOLATION OF MILK PROTEINS

Casein. A survey of the literature (9, 16, 34, 43, 44, 46, 47) shows that there is no method available to separate casein from milk which would be suitable for physico-chemical studies, if the results are to be interpreted in terms of the casein as it exists in milk. Several attempts were made to devise a procedure for isolating casein which is very low in fat and in a non-denatured form. The following was found to be a very suitable procedure: Raw skim milk, separated at 32° C., cooled to room temperature, was passed twice through a Sharples supercentrifuge at 36,000 rpm. The milk fat content of the skim milk as determined by the Babcock method was thus reduced from 0.015 per cent to 0.01 per cent to 0.00 per cent successively. The protein concentration decreased from 3.3 per cent to 2.8 per cent. The supercentrifuged skim milk was held overnight at 2° C. and the casein was precipitated by adding 0.1 N HCl according to the recommendations of Van Slyke and Baker (47). After complete sedimentation of the casein, the whey was filtered off by suction in a large Buchner funnel over filter paper. The casein then was washed thoroughly twice with cold distilled water acidified with HCl to pH 4.6, and three more times with cold distilled water. The casein was filtered by suction after each washing, then the moist casein was spread out in a glass dish and placed in the ice room at -10° C. overnight. The frozen casein was then dehydrated with the aid of an electric fan. After three successive freezings and dehydrations, the semi-dry casein contained about 41.5 per cent total solids and 40.2 per cent protein. The fat content as determined by the Mojonnier method was 0.09 per cent calculated on the dry basis. The casein obtained was in the form of white fluffy particles which were easily soluble in calcium hydroxide and had a very high foaming capacity.

Calcium caseinate solutions were prepared according to Palmer's method (27) with a slight modification. The casein was ground with sufficient calcium hydroxide powder to bring the solution to pH 6.6 (instead of pH 8.0 and subsequent neutralization to pH 6.6 with phosphoric acid). The concentrated casein solutions (about 3.3 per cent) thus obtained were diluted with distilled water and phosphate buffer solution (Sorensen) to give the desired concentration.

Lactoglobulin. The lactoglobulin fraction in separated milk is about 0.5 grams per liter. It is obviously desirable, therefore, to employ some simple means of concentrating the whey before submitting it to procedures involving salting out and dialysis. Palmer and Richardson (28) and Palmer (26), described a simple and safe method by which the whey was concentrated by freezing. The concentrated whey (3:1) thus obtained was half-saturated with 18 grams of anhydrous sodium sulfate per 100 ml. of whey and left for eight hours to precipitate at room temperature (the brownish white precipitate concentrated at the surface of the container). The lactoglobulin was filtered, washed with half-saturated solution of sodium sulfate, dissolved in distilled water, dialyzed against ice water and then against 3 per cent sodium chloride. The solution further concentrated by pervaporation (17), was stored in sterilized bottles at 2° C. and was used within 10 days of its preparation. The interference of the fat with the foaming of lactoglobulin led to the use of supercentrifuged rennet whey or the acid whey obtained from supercentrifuged skim milk. It was found necessary also in some instances to extract the concentrated lactoglobulin solution with hexane.

Lactalbumin. The whey filtrate, after removing the globulin fraction, was warmed to 35° C. and sodium sulfate added to bring its final concentration up to 36 grams salt per 100 ml. (26). The white precipitate filtered off at 35° C. was dissolved in distilled water and dialyzed against running water at 2° C. until free from sulfate ion. The crystal clear lactalbumin solution was further concentrated by pervaporation and stored in a sterilized bottle at 2° C. for use within 10 days of its preparation. According to reports by Pedersen (29), one can assume that the lactalbumin thus obtained was composed of the α and β fractions.

RESULTS

Foaming Properties of Casein

Effect of concentration. The effect of a progressive increase in concentration of casein on the foaming stabilities of its solutions is shown in figure 3. In both the presence (2.7 per cent) and the comparative absence of fat (0.06 per cent dry basis), it is to be observed that with increasing concentration of protein, the stability of the foam increases to a maximum then decreases again. The foam heights (50) on the contrary, were observed to reach a maximum value with no further decrease with increasing concentrations. The surface tension decreases to a constant value. In these experiments, the pH of the solutions was kept at 6.6 by the use of a phosphate buffer. The zone of maximum foam stability seems to be a function of the amount of fat present in the solution and the temperature at which the foaming properties are tested. Low temperature and low fat content favor the stability of calcium caseinate foam.

Effect of temperature. Preliminary results indicated that the foam height, foam stability and surface tension of calcium caseinate solutions all decreased progressively as the temperature increased from 5 to 55° C. The greatest effect of temperature was found to be on the stability of the foam. Casein prepared by the Hammarsten or Van Slyke and Baker methods did not give stable foams which might be due to partial denaturation of the protein during the method of preparation. As an example, the half-volume time of the foams of 2.8 per cent calcium caseinate solution (Van Slyke and Baker methods) was 310 seconds at 5° C. and 13 seconds at 55° C. Casein prepared by Cohn and Hendry's method contained 2.7 per cent fat on the dry basis (Mojonnier). The foam stability of this casein was low due to the interference of fat; the half-volume time of 2.4 per cent calcium

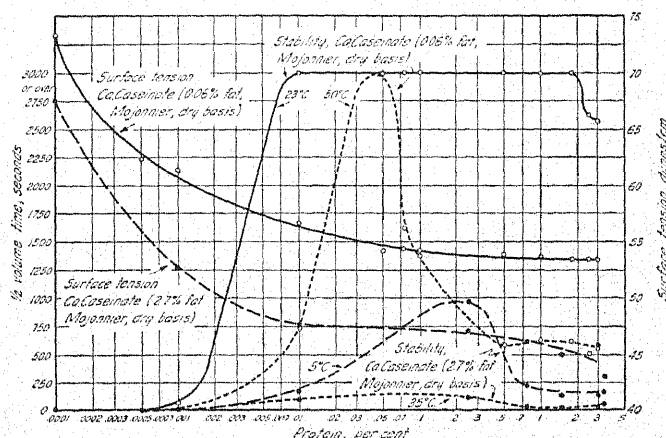


FIG. 3. The effect of concentration on the foam stabilities and surface tension of Ca caseinate solutions.

caseinate solution was about 220 seconds at 5° C. and 17 seconds at 55° C. It was also observed that elution of casein by Ansbacher, Flanigan and Supplee's method (1) did not decrease the foaming capacity or the stability of the calcium caseinate solution. In some cases, an actual increase with the eluted casein was observed which was correlated with a decrease in the fat content of the casein. Figure 4 shows a sample of the results obtained.

Calcium caseinate solutions prepared by the new method described in this paper from ultracentrifuged separated milk yield foams of greater capacity and stability (figure 5). Our data have shown that the surface tension of a 1.1 per cent calcium caseinate solution decreases only slightly with an increase in temperature (from 54.2 dynes at 5° C. to 50 dynes at 55° C.) and the foam heights are approximately proportional to the volume of air introduced at all temperatures (50). The greatest change is observed in the stability measurement, which increases slightly with temperature up to

22° C., then starts to decline slowly at first up to 35° C., and rapidly afterwards to 55° C. This great improvement in the stability of the foam is doubtless related to the lack of denaturation of the protein as well as the low fat content of casein prepared by this method.

Effect of fat. The addition of 0.015 per cent milk fat emulsion to the calcium caseinate solution decreases the foam height and the stability of the

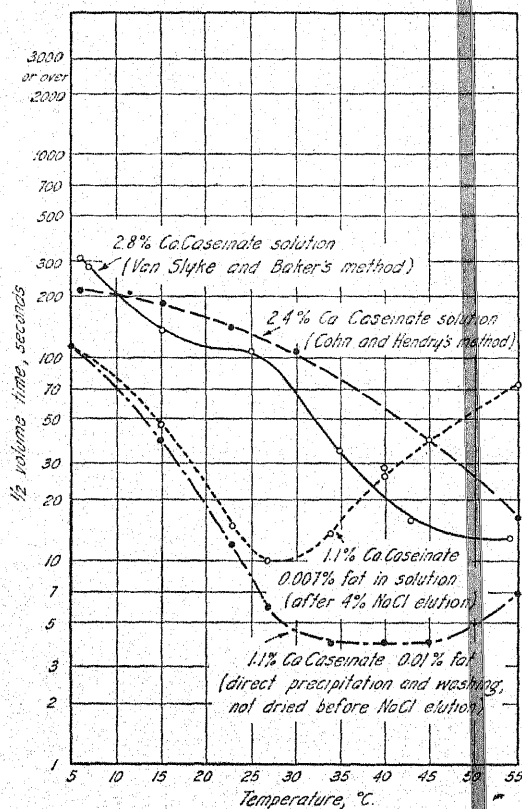


FIG. 4. The effect of methods of preparing casein on the foam stabilities of caseinate solutions.

foam to a marked extent. The reduction of the stability of the foam is greatest at temperatures above 15° C. as shown in figure 5.

The fat emulsion was prepared by emulsifying about 1 per cent milk fat in dilute phosphate buffer at 60° C. followed by cooling in ice water. The emulsion showed marked stability due to the decrease of the fat/solution interfacial tension by the salts.³

³ See Harkins, W. D., Some aspects of surface chemistry fundamental for biology, Jour. Chem. Physics., 6: 171, 1938.

FOAMING QUALITIES OF LACTOGLOBULIN

Effect of concentration. Lactoglobulins prepared from supercentrifuged rennet whey do not give stable foams. The effect of increasing the lactoglobulin concentration from 0.0001 to 0.75 per cent on the foam stability was tested at 25° C. and 50° C. The results plotted in figure 6 indicate that the foaming capacity increases slightly with concentration at 25° C. The results obtained at 50° C. show that there is a subsequent decrease in foam

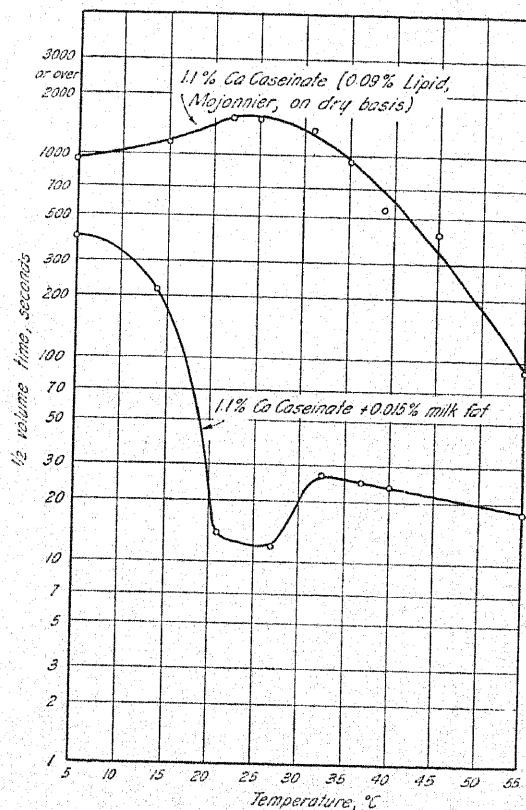


FIG. 5. The foam stabilities of Ca caseinate solutions of casein prepared by the present method and the effect of adding milk fat emulsion.

stability with an increase in concentration. The surface tension continues to decrease as the concentration increases. The foam stability follows the same pattern as that reported for calcium caseinate. On the whole, it is quite clear that lactoglobulin has very poor foaming qualities. This may be attributed to the presence of a lipid material associated with its protein molecule (10, 24).

Effect of temperature and fat content. Table 1 shows that 0.05 per cent lactoglobulin solutions (the concentration normally present in milk) do not

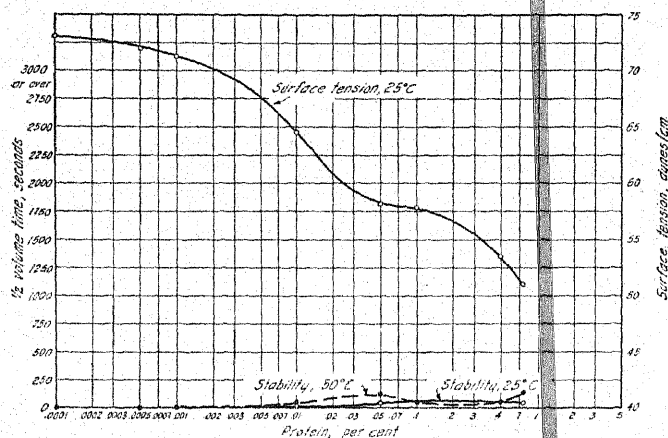


FIG. 6. The effect of concentration on the foam stabilities and surface tension of lactoglobulin solutions.

form stable foams at any temperature tested, when the solution contained about 0.006 per cent fat. As the fat content was decreased to 0.001 per cent by extracting the same solution twice with equal volumes of hexane, the foam height and the stability increase at all temperatures tested, the increase being greater at temperatures above 20° C. Lactoglobulin solutions were prepared in phosphate buffer solutions at pH 6.6.

THE FOAMING PROPERTIES OF LACTALBUMIN

Effect of concentration. The effect of increasing the concentration of lactalbumin at 25° C. and 50° C. is illustrated in figure 7. As the concentration increases from 0 to 1 per cent the foam stabilities increase to maximum values and remain unchanged (the foam heights were observed also to reach maximum values (50)). The surface tension continues to decrease

TABLE 1

Foaming properties of lactoglobulin. Effect of temperature on 0.05% lactoglobulin solutions of different fat contents

0.05% lactoglobulin precipitated once from rennet whey (0.0065% fat in the solution)				Same globulin solution extracted twice with hexane (0.001% fat in the solution)			
Temperature	Surface tension	Foam height	1/2 volume time	Temperature	Surface tension	Foam height	1/2 volume time
°C.	dynes	mm.	sec.	°C.	dynes	mm.	sec.
5.0	54.4	45	5	6	49.0	120	20
15.0	51.6	25	5	10	49.1	115	19
22.5	49.4	8	2	17	100	17
32.0	48.2	40	4	20	48.0	120	21
43.0	47.5	190	20	32	49.3	220	50
52.0	47.1	260	72	40	46.9	260	380

to a minimum value. It is of interest to note that lactalbumin is more surface-active with regard to foaming, at 50° C. than at 25° C., as evidenced by the fact that less protein concentration is required to support a stable form at the former temperature.

Effect of temperature. The results illustrated in figure 8 show that a 0.047 per cent lactalbumin solution forms a much more stable foam than a corresponding concentration of lactoglobulin solution. The half-volume time increases with the rise of temperature from 5° C. to 23° C. where it reaches the maximum limits set for this study and remains so up to 50° C. When the protein concentration is increased to 0.18 per cent, the foam stability exceeds the limits at all temperatures. The foam volumes were found to be equal to the volume of air forced through the solutions (50).

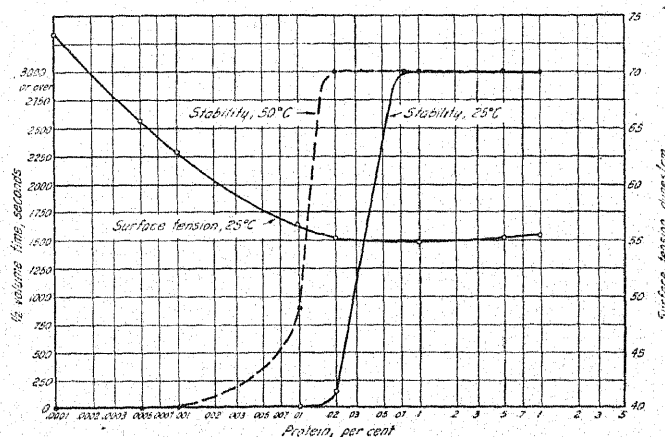


FIG. 7. The effect of concentration on the foam stabilities and surface tension of lactalbumin solutions.

Effect of fat. The effect of adding different amounts of milk fat emulsion stabilized in dilute phosphate buffer is quite interesting—figure 8. The addition of 0.0005 per cent fat to a 0.05 per cent lactalbumin solution decreases the stability of the foam greatly at temperatures below 25° C. and to a less extent at 30.5° C. but has no effect above 38° C. When the fat content is further increased to 0.005 per cent in 0.42 per cent lactalbumin solution, the reduction in the foaming properties is much greater at all temperatures. These changes are accompanied by a lower surface tension. A further increase of the fat content to 0.5 per cent in the 0.05 per cent lactalbumin solution, decreases the foam measurements (and surface tension (50)) to still lower limits. It is clear then that the foaming capacity of a lactalbumin solution is a function of the amount of fat present and the temperatures at which the foaming properties are measured. The inhibiting effect of milk fat is reduced at temperatures above its melting-point. The

next experiments were designed to study the effect of increasing the albumin content in a solution, the foaming properties of which were greatly inhibited by the presence of a relatively high fat concentration. As shown in figure 8, increasing the concentration of albumin from 0.05 to 0.5 per cent in a solution containing 0.5 per cent milk fat improves the stability of the foam only slightly at temperatures below 39° C. but to a great extent above that tem-

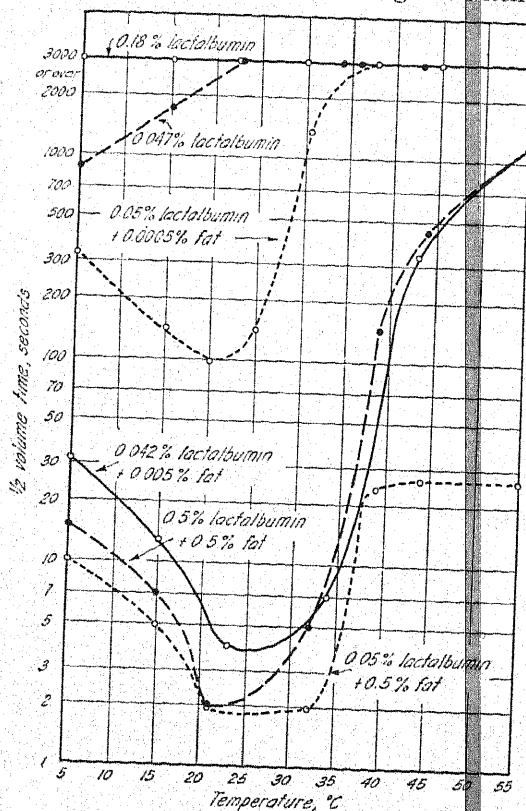


FIG. 8. The foam stabilities of lactalbumin solutions with different amounts of milk fat emulsion added.

perature. The surface tension values are about the same in both solutions (47.3–47.0 dynes respectively (50)).

DISCUSSION

The experimental results have shown that the foaming properties of solutions containing surface-active constituents of milk vary markedly with a number of factors depending on the chemical nature and the amount of materials present in the solution, and the physical conditions under which the measurements are obtained. The variations in the foaming properties

of milk proteins can be discussed best if only the stability of the foam is considered, since in some instances the foam heights are equal while the stabilities are quite different.

The effect of increasing the concentration of calcium caseinate or lactoglobulin in buffered solutions at pH 6.6 results in a progressive increase in the foam stability to a maximum followed by a subsequent decrease. The latter decrease is not noticed in the case of lactalbumin solutions up to 1 per cent concentration. The explanation of these facts cannot be based on changes of surface tension⁴ or viscosity, a conclusion which has been reached by several investigators (11, 23, 30, 49). The same phenomenon of the relation of concentration to the foam stability, observed by Bartsch (2) on simple organic compounds, was explained on the boundary heterogeneity theory. Thus, the foam stability increases when the air/solution interface becomes heterogeneous with respect to the solvent and the solute (a condition which is not present in very dilute or concentrated solutions). Talmud (45) relates the maximum foam stability to the concentration that allows for maximal hydration at the interface. At the optimum concentration the organic molecules are oriented, inclining at a certain angle to the surface and interlacing with each other to form a kind of gel structure or a film with the highest tensile strength. Therefore, in order to discuss the stability of protein foams, consideration should be given to the micellar size, solubility, orientation, and the degree of hydration, when such information is available. Lactalbumin, being highly soluble in water and having a low molecular weight (17,500-39,000) (29) might well be placed with the soluble compounds in Bartsch's classification of the foaming substances (2). On the other hand, casein and lactoglobulin with a low solubility in water and with high micellar weights (average 87,000 and 140,000 respectively) (29) may be placed with insoluble substances in the same classification.

The effect of temperature on the foam stability of milk protein solutions may be explained on the same principle; *i.e.*, there is an optimum molecular size and degree of hydration that permits the formation of a lamella which has a high tensile strength. For example, a solution of calcium caseinate (figure 5) forms a foam that has a half-volume time of approximately 1520 seconds at 22° C. and 90 seconds at 55° C. This rapid decrease in foam stability at temperatures above 35° C. can be correlated with the fact observed by Svedberg, Carpenter and Carpenter (44) that the micellar size of calcium caseinate is doubled by warming its solution to 40° C. These authors believe that the heating causes a polymerization or association of the molecules to form larger aggregates. Such an increase in size along with the dehydration of casein upon heating (23, 48) would seem to be among the important

⁴ K. G. A. Parkhurst, in a recent paper (Trans. Faraday Soc., 37: 496-505, 1941), stated that it is not low surface tension *per se* that ensures foam stability but the nature of the adsorbed layer giving rise to the lowering of surface tension.

factors responsible for the decrease of foam stability of a calcium caseinate solution heated to 55° C. Further evidence on the importance of the particle size of calcium caseinate to the foam stability of its solution can be obtained from correlating the findings of Carpenter (7) with the foam stability obtained in this study. According to Carpenter, the molecular weight of casein in a 1.5 per cent solution is 96,000 at pH 6.8 (M/60 phosphate buffer), but on dilution to less than 0.7 per cent, the micelle dissociates to one-third the initial weight, namely 32,000. This dissociation is completely reversible and thus confirms Sorenson's conception (41, 42) of a protein as a complex undergoing reversible association and dissociation. This decrease in micellar size on dilution is accompanied by changes in physical properties, as for example, an increase in specific rotation. The foam stability of different concentrations shown in figure 3 indicates that as the micellar weight of calcium caseinate decreases by dilution, the foam stability increases. For example, at 50° C. the half-volume times are 630 and 1370 seconds, corresponding to concentrations of 1.0 and 0.1 per cent respectively. At a concentration of 0.05 per cent, a calcium caseinate solution (prepared from unaltered casein) gives an apparently clear solution that has about the same micellar size (7) and foam stability as a 0.05 per cent lactalbumin solution. Likewise, sodium caseinate appears to have a smaller micellar size than calcium caseinate (43b) and the results, not reported in this paper, show that it has higher foam stability.

It is quite important to note that none of the casein solutions, at concentrations above 0.5 per cent, supports a stable foam at temperatures above 43° C., where the skim milk forms a very stable foam.

No data are available on the changes of particle weight or the degree of hydration of lactalbumin or lactoglobulin as the temperature is increased from 5 to 55° C. The facts reported in table 1 and figure 8 indicate that both protein solutions have greater stability at higher temperatures and, by analogy, it may be reasoned that the conditions favorable for forming lamellae with higher tensile strength and greater stability are better fulfilled at these temperatures. One of the present trends of thought in protein chemistry (21) suggests that the larger protein molecules consist of small primary protein units linked into a secondary structure by means of carbohydrate, phospholipid, nucleic acid or polyvalent inorganic ions, and that these units are subject to association and dissociation under different conditions (7, 21, 41, 42). It may be of value to consider the foaming stability as one of the physical properties that reflects such an association and dissociation as pointed out in discussing the foaming properties of calcium caseinate solutions.

The effect of adding small amounts of milk fat emulsion in phosphate buffer to milk protein solutions is quite interesting. The data indicate that the foam stabilities of lactalbumin solutions are reduced to a greater extent

by the fat at temperatures below the melting point of the milk fat (figure 8). The reverse is observed in calcium caseinate solutions (figure 5). A 0.05 per cent lactoglobulin solution does not give a stable foam in the presence of as little as 0.0065 per cent fat, yet the stability of the foam is greater at temperatures beyond the melting point of milk fat (table 1). The action of milk fat on the foaming properties will be discussed in a later paper.

SUMMARY

An apparatus is described for accurate measurements of the foaming properties of milk. Experimental data have revealed that the subsidence of skim milk foams cannot be defined by a single equation at temperatures between 5 and 55° C. For this reason, a unit called a "half-volume time" has been proposed and used to compare the stabilities of static and dynamic foams. The unit can be converted by a given equation to give average life of a foam bubble, if the foam follows a logarithmic subsidence.

The major surface-active proteins of milk, including casein, α - β -lactalbumin and lactoglobulin, have been separated, purified and studied for their foaming properties. A new procedure for isolating undenatured casein with a lipid content as low as 0.06 per cent has been introduced. The method is based on super-centrifuging skim milk, precipitating the casein at a low temperature with 0.1 N HCl, washing, freezing, and dehydrating the frozen casein by pervaporation.

The foam "half-volume time" values of milk protein solutions in M/30 phosphate buffers at the normal hydrogen ion concentration of milk varied between 2 and 3000 seconds (or over), depending upon the kind of protein, concentration, method of isolating the protein, temperatures, and the amount of milk lipids present in association with the protein molecule or added in the form of an emulsion to the solution. Studies of these variables are reported and some theoretical explanations advanced.

It is shown that calcium caseinate solutions of a concentration equivalent to that of milk and prepared by the present procedure have high foam stabilities at temperatures below 40° C. Lactalbumin solutions foam well at all temperatures, but lactoglobulin solutions show no appreciable foaming. The foam depressing action of milk fat is shown to be greater at temperatures over 15° C. with calcium caseinate solutions and at temperatures lower than 35° C. with lactalbumin solutions.

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THE ROLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS.

II. WHEY, SKIMMED MILK, AND THEIR COUNTERPARTS

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REVIEW OF LITERATURE

The foaming properties of some milk products represent a unique colloidal phenomenon; that is, the foam stability decreases to a minimum, as the temperature increases, after which it again increases. The first report on the effect of temperature was made by Siedel (22). He found that the quantity of foam depends on the temperature of the milk and that the nature of the foam at low temperature is different from that at high temperature. Siedel's studies did not go beyond 35° C., but he noticed an inflection in the stability of the foam. Leete (11) and Sanmann and Ruehe (18) studied the effect of temperature on milk foam. Separated milk and milk foamed least between 20 and 30° C. according to the former, and at 27° C. according to the latter. Both investigators agreed on the increase in the foam at temperatures beyond 30° C. but disagreed on the temperature at which the maximum foaming appeared.

There has been considerable speculation as to the cause of this minimum foaming ability of milk. Rahn and Sharp (15) raised the question whether these kinks in the foam-temperature curve indicate the presence of a specific foam-producing material or of a stabilizing substance in the foam. Ansbacher and co-workers (1) reported that the foam-producing substance eluted from casein showed a critical agglomeration at the temperature of the minimum foaming. They did not present data regarding the foaming stability of this highly foaming constituent of milk. Leviton and Leighton (12) tried to explain this kink on the basis of the work of King (10), who showed that the portion of the surface of milk covered with a film of fat increased with a rise in temperature from 10 to 20° C. They postulated that the decrease in the foaming power might be explained on the basis of the increase in the tendency of the fat to spread as the temperature rose. No comment was made on the further increase of foam formation with increasing temperature.

Holm (9) explained the minimum foaming of milk as a result of variation in those properties of the solution which evidence themselves in viscosity

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changes. Davies (3) considered that the range from 20 to 30° C. represents conditions in which hydration is practically nil and the stabilizing factors, *i.e.*, the decrease in surface tension and denaturation of the proteins, have not come into play. Ansbacher *et al.* (1), however, found that the viscosity of their foam material was the same as that of water.

The authors are not aware of any published work on the foaming properties of whey.

In a previous paper (5a) the authors reviewed the existing controversy as to the foaming constituents in milk. Using the "half-volume time" for measuring the foam stabilities of the major milk proteins, it was found that calcium caseinate solutions have high foam stabilities at certain concentrations at temperatures below 40° C.; lactalbumin solutions foam well at all temperatures, but lactoglobulin solutions show no appreciable foaming properties. Foam-depressing action of milk fat was found to be greater at higher temperatures with calcium caseinate solutions, and at lower temperatures with lactalbumin solutions.

The present work deals with the preparation of synthetic whey and separated milk by combining the milk protein fractions in an attempt to duplicate the foaming properties of these products. As a result an explanation of the effect of heat on the foaming properties of whey, separated milk and milk is offered.

EXPERIMENTAL

The foam measuring apparatus, technique, and other methods used have been described in a previous paper (5a). Separated milk and rennet whey used in this work were obtained from the University Farm Creamery. The foaming properties of the solutions studied were compared at different temperatures between 5 and 55° C.

The foaming properties of whey and synthetic whey. The variation of the foam stabilities of various wheys with temperature is shown in figure 1. As the temperature increases from 5 to 55° C. the foam stability of the ordinary rennet whey (0.035 per cent fat) decreases to a minimum value between 22 and 32° C. and is followed by an enormous increase at higher temperatures. On combining solutions of lactalbumin and lactoglobulin separated from this whey, to give concentrations of 0.61 per cent and 0.05 per cent respectively in a phosphate buffer of the same pH as the whey, the foaming properties were found to be practically the same as the original whey. The type and general characteristics of the foam at low temperatures were identical with those of lactoglobulin solutions; those at temperatures above 32° C. resembled lactalbumin solutions.

In order to reduce the fat content of the whey and of the lactalbumin and lactoglobulin separated from it without the use of fat solvents the whey was supercentrifuged. The fat content of the whey was thereby decreased from 0.04 per cent to about 0.0001 per cent, and the protein decreased by

0.02 per cent. The foaming properties were greatly improved. A similar improvement in the foam height and stability were obtained with acid whey prepared from supercentrifuged skim milk (5b).

Solutions of lactalbumin and lactoglobulin (prepared from supercentrifuged whey), when combined to give the same concentration as in whey, give essentially the same foaming properties as the supercentrifuged whey itself (figure 1).

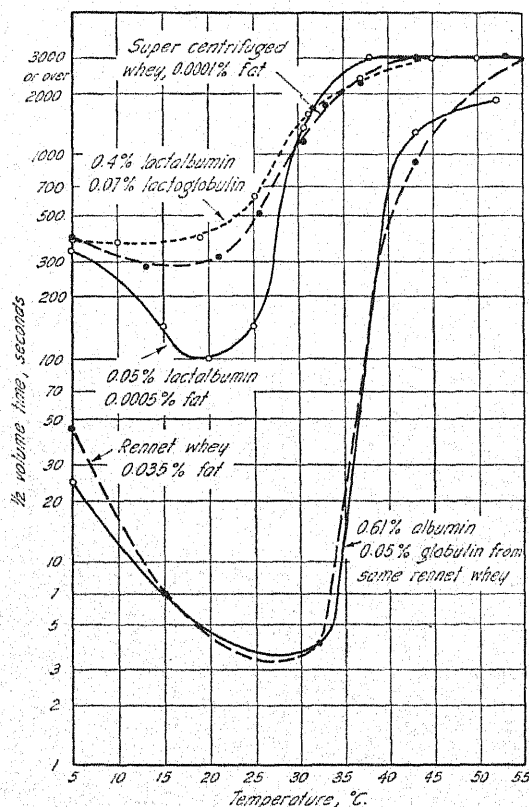


FIG. 1. The foam stabilities of whey with different fat concentrations and their duplication with lactalbumin solutions with fat or lactoglobulin added.

In a former paper (5a) it was shown that lactalbumin solutions (0.10 per cent or over) give stable foams represented by half-volume times greater than 3000 seconds at all temperatures. The present data indicate that the presence of lactoglobulin (from supercentrifuged whey) in a 0.4 per cent lactalbumin solution reduces the foam stability of the latter at lower temperatures to about 10 per cent of its original value. The addition of a milk fat emulsion in phosphate buffer to a lactalbumin solution (ratio 1:100 in solution), however, is shown to have an effect similar to the lactoglobulin.

On the other hand, when lactoglobulin, separated from ordinary rennet whey (0.035 per cent fat) is added to a 0.61 per cent lactalbumin solution, the foam stability of the latter is greatly reduced at the lower temperatures. The mixture has about the same foam stability as the original whey. The further reduction in this case undoubtedly is due to the presence of fat in the isolated lactoglobulin.

The foaming properties of separated milk. Raw separated milk (0.01 per cent fat by Babcock test) obtained from mixed herd milk forms a rela-

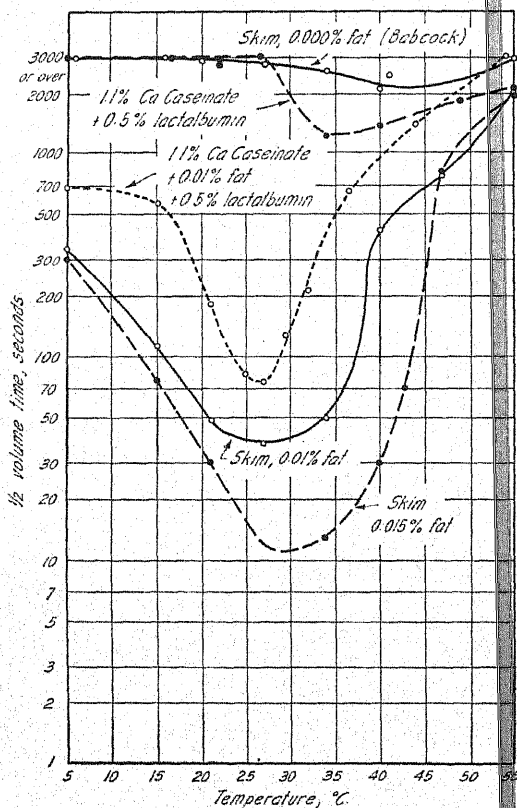


FIG. 2. The foam stabilities of skim milks with different fat content and their duplication with Ca caseinate + lactalbumin with and without milk fat added.

tively high stable foam at 5° C. On increasing the temperature to 25° C., both the foaming capacity and the half-volume time of the foam are reduced from 200 mm. and 429 seconds to 170 mm. and 21 seconds respectively. By increasing the temperature to 55° C. the foaming properties are improved again until a second maximum, much higher than the first, is reached (figure 2). The type of the foam changes also with the increase in temperature; that is, from relatively large and loose, to small, compact foam cells.

The effect of decreasing the fat content by passing the separated milk through a Sharples Supercentrifuge (36,000 rpm.) is shown in figure 2. As the fat content decreased from 0.015 to practically zero per cent (Babcock test), the skim milk showed remarkable foaming properties at all temperatures. The half-volume time of the foam at 27° C. increased from about 37 seconds (original) to about 2800 seconds (super-skimmed). There is no minimum foaming of supercentrifuged skim milk, which indicates that the

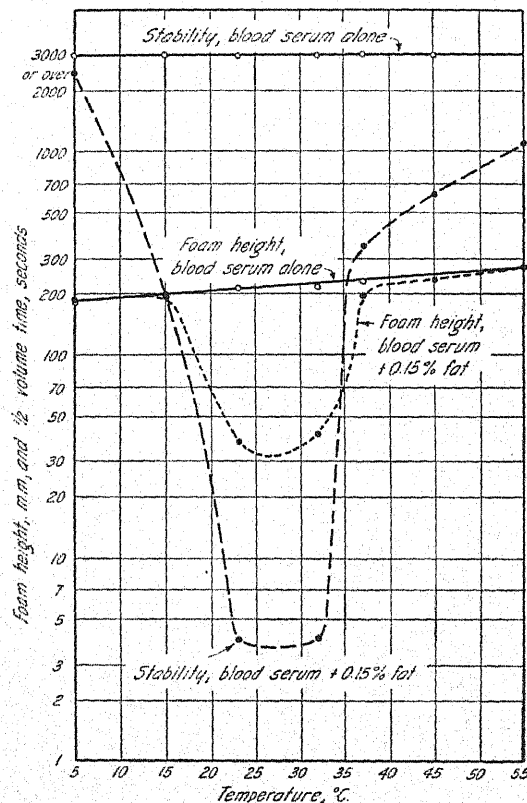


FIG. 3. The effect of adding fat emulsion on the foaming properties of "horse blood serum."

tendency of ordinary separator milk to give minimum foam values at room temperature is due to the presence of the fat globules.

Effect of adding fat emulsion to blood serum. To confirm further that the minimum foaming of separated milk is mainly due to the presence of fat globules, horse blood serum, obtained by defibrinating fresh blood with glass beads, was tested for foaming properties before and after the addition of a 0.15 per cent milk fat emulsion in phosphate buffer. The results reported in figure 3 show that the "half-volume time" is reduced by the presence

of fat from over 3,000 to 4 seconds at room temperature. Further increases or decreases in temperature improve both the foaming capacity and stability.

Comparison of the separated milk and whey foams. In comparing the foam-temperature curves of the separated milk (figure 2) and whey (figure 1) in the presence and absence of fat, it becomes evident that skim milk has a relatively higher foam stability in the temperature range from 5 to 35° C. than the corresponding whey. The main difference in the chemical composition of the skim milk as compared with the whey lies in the absence of casein from the latter. Therefore, it is reasonable to assume that the presence of calcium caseinate accounts for the greater stability of skim milk foam at lower temperatures.

Foaming of a reconstructed separated milk. On the basis of the previous findings that the constituents responsible for the foaming of the whey are lactalbumin and a lipid fraction, it was anticipated that by adding non-

TABLE 1

The effect of the addition of 0.05 per cent lactoglobulin on the foaming properties of a 1.1 per cent calcium caseinate (0.09 per cent fat, dry basis) solution*

Temperature	Surface tension		Foam height		Half-volume time	
	Calcium caseinate	Ca caseinate plus lactoglobulin	Calcium caseinate	Ca caseinate plus lactoglobulin	Calcium caseinate	Ca caseinate plus lactoglobulin
°C.	dynes/cm.	dynes/cm.	mm.	mm.	sec.	sec.
5	54.2	54.6	201	200	935	850
15	53.7	52.8	213	215	1170	1030
25	51.0	51.6	220	218	1500	1525
35	51.0	50.6	247	241	950	595
45	50.2	49.4	245	235	423	182

* Prepared from twice supercentrifuged skim milk.

denatured calcium caseinate to these two constituents a solution would be obtained having the foaming properties of skim milk. The results obtained and shown in figure 2 confirmed that idea. It is possible to get an approximate duplication of the foaming characteristics of skim milk (0.01 per cent fat) with a solution containing 1.1 per cent calcium caseinate, 0.5 per cent lactalbumin, and 0.01 per cent milk fat emulsion in phosphate buffer. The increase observed in the foam stability of the synthesized solution can be accounted for by the absence of the phospholipid fraction which normally surrounds the fat globules. The effect of the fat globule membrane in determining the depressing action of the fat on the foam was previously studied (5). A buffered solution (pH 6.6) of 1.1 per cent calcium caseinate and 0.5 per cent lactalbumin gives approximately the same foam stabilities as the supercentrifuged skim milk (0.00 per cent fat by the Babcock test). Both the reconstituted and the supercentrifuged skim milk form foams of remarkable stability at all temperatures. The "half-volume times" of the foams were over 1000 seconds at every temperature used.

Table 1 shows that the addition of 0.05 per cent lactoglobulin to a calcium caseinate solution does not change the foaming properties of calcium caseinate appreciably except at the higher temperatures at which the fat is liquid. The lower foam stability of the mixture at 35° and 45° C. is to be expected in view of the additional lipid material contributed by the lactoglobulin.

From these results it is concluded that the foaming properties of skim milk are due to the presence of calcium caseinate, lactalbumin and milk fat. A solution containing 0.6 per cent lactalbumin, 0.05 per cent lactoglobulin, and 2.7 per cent calcium caseinate (which is prepared from casein extracted with hot alcohol, ether and dried) however, does not have the same foaming properties as skim milk (Table 2). The results support the belief that the physical properties of the casein treated with alcohol and ether are altered.

TABLE 2

Foaming properties of synthetic solutions containing casein which has been treated with alcohol and ether, in comparison to skim milk

No.	Sample	30° C.		43° C.		55° C.	
		Foam height	$\frac{1}{2}$ volume time	Foam height	$\frac{1}{2}$ volume time	Foam height	$\frac{1}{2}$ volume time
		mm.	sec.	mm.	sec.	mm.	sec.
1.	2.7% Ca caseinate solution (casein extracted with alcohol and ether and dried)	182	49	190	16	160	13
2.	2.8% Ca caseinate from casein No. 1, plus 0.6% lactalbumin and 0.05% lactoglobulin in solution	140	18	150	17	205	54
3.	Skim milk + 0.018% fat	170	22	240	400	260	2005

The relation of "agglutinin" to the foaming of separated milk. Sharp and Krukovsky (19) attributed the high foaming properties of skim milk separated at 50° C. to the presence of an agglutinin and the poor foaming of that separated at 5° C. to the absence of agglutinin. This work is not in agreement with the present findings that calcium caseinate and lactalbumin are the chief foaming proteins in skim milk. Consequently, their work was repeated with the hope of finding the reason for such a difference in the foaming of separated milk obtained at 5° C. and 50° C.

Preliminary results obtained by separating raw milk at 5° C. and 32° C. indicated that the skim milk samples separated at 5° C. behaved abnormally with regard to the foaming properties; that is, the stability of the foam was reduced at 5° C. and at 55° C., but was increased at room temperature. Further research revealed that the resulting complications arose from two factors: 1) a slight development of rancidity which is probably due to centri-

TABLE 3

Effect of the temperature of separation on the foaming ability of the recombined milks

4.25 per cent pasteurized milk separated at two temperatures—at 5° C. (skim milk 0.11 per cent fat and 22.6 per cent cream) and at 53° C. (skim milk 0.15 per cent fat and 23.5 per cent cream). All the possible combinations of 4.25 per cent milks were prepared—the foaming properties of the original raw milk and the skim milks were tested at 3 different temperatures.

Sample	5° C.				25° C.				50° C.			
	Surface tension	Foam height	$\frac{1}{2}$ volume time	dynes/cm.	Surface tension	Foam height	$\frac{1}{2}$ volume time	dynes/cm.	Surface tension	Foam height	$\frac{1}{2}$ volume time	sec.
(1) Raw milk	53.0	204	47	46.9	35	35	5	270	1800	
(2) Pasteurized milk	53.6	200	34	47.3	22	22	4	288	3000	
(3) 53° C. skim 0.015% fat	54.9	200	460	53.5	180	19	19	49.9	280	3000	
(4) 5° C. skim 0.11% fat	54.8	200	128	48.9	40	4	4	46.9	275	3000	
(5) 53° C. skim 0.11% fat	55.0	202	133	48.6	20	3	3	46.1	276	3000	
(6) 53° C. skim + 53° C. cream	53.5	198	27	47.5	30	4	4	287	3000	
(7) 5° C. skim + 5° C. cream	53.5	200	30	47.4	26	4	4	284	3000	
(8) 53° C. skim + 5° C. cream	53.6	200	30	47.8	23	4	4	286	3000	
(9) 5° C. skim + 53° C. cream	53.2	197	29	47.6	26	4	4	284	3000	

Cream Volume Measurement

Sample	Cream volume %					
	Hours					
	0	2½	6½	8½	20	
(2) Pasteurized milk	0	17.0	16.5	16.0	15	
(6) 53° C. skim + 53° C. cream	0	15.0	14.75	14.75	14	
(7) 53° C. skim + 5° C. cream	0	15.0	15.0	14.5	14	
(8) 53° C. skim + 5° C. cream	0	18.0	17.0	16.5	15	
(9) 5° C. skim + 53° C. cream	0	3.5	6.5	8.0	9	

fuging milk at low temperatures:² 2) the higher fat content in the skim milk due to inefficient separation at low temperatures.

The data on the effect of rancidity and homogenization on milk foam will be reported in a later paper. Our results show that a slight development of rancidity results in a marked decrease in the stability of milk foam at 5° and 50° C., while it increases the foam stability at temperatures between 10 and 30° C.

According to these facts, the assumption was made that if the work of Sharp and Krukovsky were to be repeated with pasteurized milk (61.5° C. for 30 minutes) and the fat were to be increased to correspond to that separated at low temperature, then both skim milks would have the same foaming properties. The assumption was borne out by the experimental results reported in table 3.

These facts do not exclude the possibility of the presence of an agglutinin in milk because the measurements obtained on the cream volume of reconstituted milks agree with the results of Sharp and Krukovsky. However, the data indicate clearly that the agglutinin is not one of the constituents which determine the foaming properties of milk.

DISCUSSION

The data presented in figure 1 show the similarity in the foaming properties of whey and synthetic solutions containing lactalbumin and lactoglobulin in their normal concentration. They show also that the addition of small amounts of milk fat to lactalbumin solutions gives foaming properties similar to whey. In other words, the reduction in the foam stability of lactalbumin solutions that results from adding lactoglobulin is probably due to the presence of small amounts of fat associated with lactoglobulin. Therefore, it is reasonable to conclude that lactalbumin and milk fat are the major constituents that determine the foaming properties of whey.

In figure 2 it is shown that the minimum foaming of separated milk at room temperature is due to the presence of fat in the separated milk. When the fat globules were largely removed by passing that separated milk through a supercentrifuge, it showed about the same foaming properties at all temperatures tested. It has been shown also that the essential foaming characteristics of supercentrifuged skim milk can be duplicated with a solution of calcium caseinate plus lactalbumin, and that the addition of 0.01 per cent milk fat to this solution decreases its foam stability to a minimum at 27° C. Similar observations on horse blood serum are reported in figure 3. These results are taken to indicate that the presence of fat globules in biological

² Sharp and Tomasi (21), and Doan (4) have also reported on the development of rancidity in samples separated at low temperatures and pointed out that the action of centrifugal separation is somewhat similar to homogenization in that it stimulates hydrolysis of the fat in the resulting cream.

fluids reduces their foaming properties to a great extent at certain temperatures, a fact which may prove of value in testing for lipemia.

To explain the foaming properties of skim milk or milk, we are led to the belief that calcium caseinate is the foaming substance of milk at low temperatures, while lactalbumin is the substance contributing to the foam at temperatures above the melting point of milk fat. This conclusion is supported by the experimental data presented and deduced from the following considerations.

(a) A calcium caseinate solution does not form a stable foam at temperatures above 21° C. in the presence of 0.015 per cent fat (5a). At 50° C. the "half-volume time" of such a solution is of the order of 20 seconds, while that of the ordinary skim milk or milk is over 1,000 seconds.

(b) A lactalbumin solution in the presence of 0.005 per cent fat does not form a stable foam at temperatures below 34° C. but forms a stable foam at higher temperatures corresponding to those of milk and of skim milk (5a).

TABLE 4

No. of times through separator	Sample	Protein			
		Total	Cassin	Heat coagulable	Non-heat coagulable
		%	%	%	%
2	Original milk	3.89	3.05	0.40	0.44
	Foam	4.19	3.35	0.40	0.44
	Difference	0.30	0.30	0.0	0.0
5	Original milk	3.69	2.89	0.37	0.43
	Foam	4.00	3.20	0.35	0.45
	Difference	0.31	0.31	-0.02	+0.02
8	Original milk	3.57	2.77	0.37	0.43
	Foam	3.82	3.00	0.39	0.43
	Difference	0.25	0.23	0.02	0.0

(c) On mixing solutions of calcium caseinate and lactalbumin with or without the addition of fat, the essential characteristics of the foaming properties of the corresponding separated milk can be duplicated (figure 2). On the other hand, the addition of lactoglobulin to a calcium caseinate solution does not alter the foaming properties of the latter to any great extent (table 1).

(d) At temperatures below 30° C., skim milk gives a more stable foam than the acid or rennet wheys separated from it. This is due to the absence of calcium caseinate from the whey.

The conclusion is contrary to the prevailing ideas in the dairy field, but it is further justified by the data of other workers. Sharp, Myers and Guthrie (20) investigated the accumulation of protein in the foam of skim milk and came to the conclusion that "there is no preferential accumulation

of any major protein fraction in the foam." Table 4, compiled from their data, indicates that there is a preferential adsorption of casein in the foam at 10° C. which is in complete agreement with the present conclusion.

The preferential adsorption of the substance having the highest surface activity at the air solution interface is in accord with thermodynamical principles and has been reported by many investigators (2, 6, 7, 8, 16, 23, 24). Other workers have found this principle useful in biochemical studies (13, 14).

Ansbacher, Flanigan and Supplee (1) eluted commercial casein with sodium chloride and claimed that they obtained the foaming substances from casein, since the eluted casein did not foam appreciably. They did not, however, present data on this foaming substance or of the casein before and after elution. In a previous paper (5a) we showed that when casein is eluted with 4 per cent NaCl at pH 4.2, the foaming properties of the calcium caseinate prepared were not reduced. Considering that casein at its isoelectric point is soluble in 0.115 N NaCl solutions to the extent of 3.46 gm. per liter (17) and that casein has its maximum foaming capacity at a concentration of about 0.05 per cent, the complications in the studies of these workers may be realized.

Another attempt to assign the foaming properties of separated milk to a minor fraction of its proteins has been reported recently by Sharp and Krukovsky (19). These workers reported two paradoxical ideas; while they did not think that less efficient separation and consequently higher fat content in the skim milk separated at low temperature was the cause of its poor foaming properties, they reported that when this separated milk was extracted with petroleum ether its foaming properties then approached those of the high temperature separated milk. This result would seem to indicate the presence of an anti-foamer (lipoid) prior to extraction but not the absence of an agglutinin. Our results show the "agglutinin" is not important in the foaming of separated milk.

SUMMARY

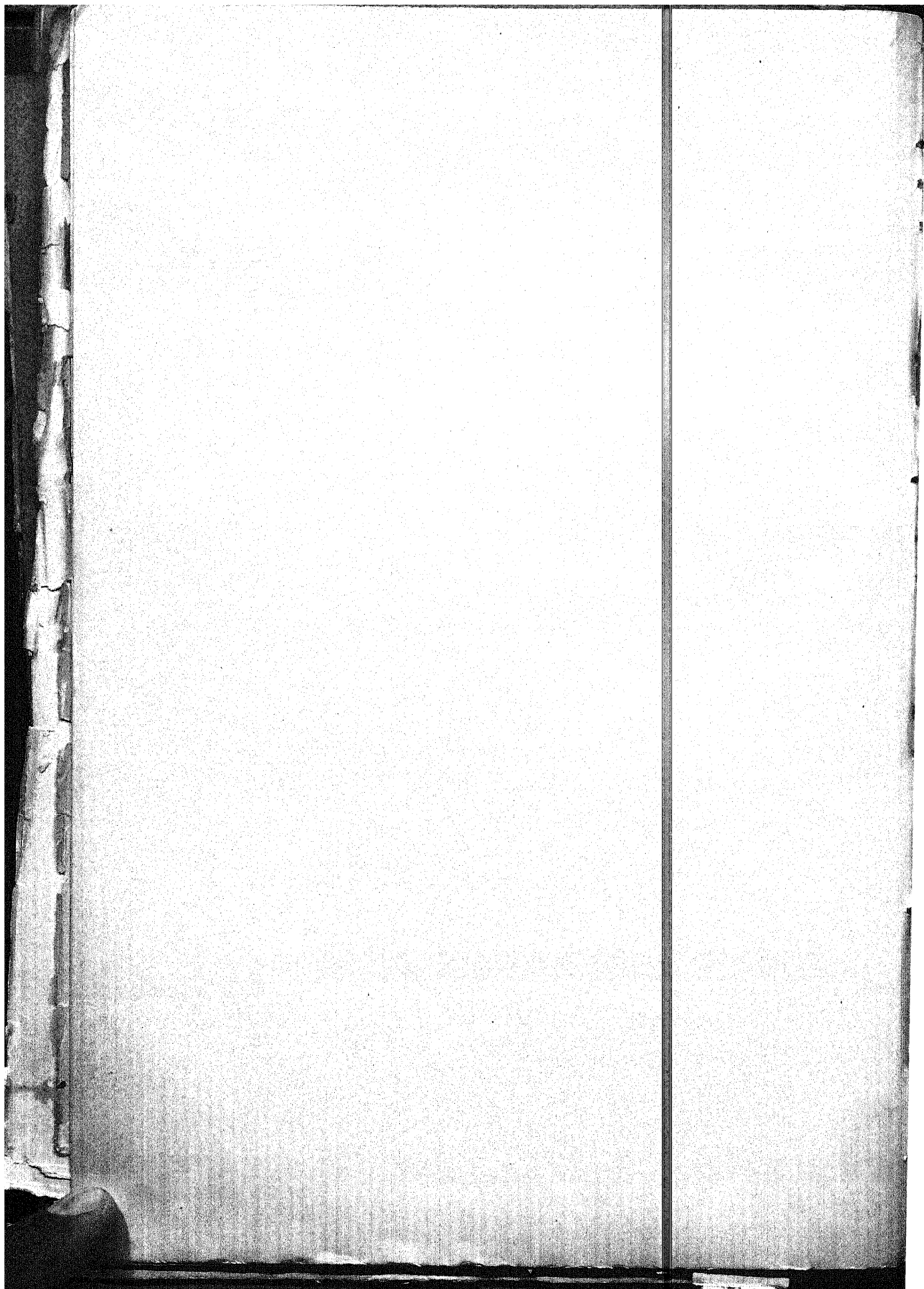
Synthetic solutions of milk proteins and fat have been prepared which duplicate the essential foaming characteristics of whey and skim milk. Lactalbumin and milk fat were found to be the constituents that influence the foaming of whey. The constituents in milk or skim milk that determine its foaming properties are shown to be calcium caseinate, milk fat and lactalbumin. It is considered that calcium caseinate is preferentially adsorbed at the air/liquid interface at temperatures below the melting point of milk fat, lactalbumin being adsorbed at higher temperatures. The presence of milk fat globules is shown to be responsible for the minimum foaming of separated milk at certain temperatures. The addition of milk fat emulsion to blood serum brings about a minimum foaming at room temperatures.

Previous work which is not in agreement with the present conclusion is analyzed in the light of the present studies.

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A STUDY OF METHODS OF OBTAINING MILK SAMPLES FOR ESTIMATING MILK FAT BY THE MOJONNIER METHOD

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Wide differences in the amounts of milk fat were obtained from some samples of the same milk by the Babcock and by the Mojonnier methods. An examination of the data revealed that the method of weighing the samples of milk and of transferring them into the extraction flask by the Mojonnier method was responsible for these abnormal variations.

Though not recognized as official, the Mojonnier method is used extensively in dairy laboratories in this country instead of the Røese-Gottlieb method (1). Some essential details of the technic for obtaining the sample of milk are lacking in the Røese-Gottlieb method (1) and three different technics are used in the Mojonnier procedure (3, 4).

The writers were unable to find any data in the literature on this subject, except a statement by Dahlberg, Holm and Troy (2) which is quoted. "It was thought that the measuring of samples would introduce slight errors and that fat might rise in the weighing pipette during the time required for weighing to such an extent that extra rich milk might remain in the pipette after the sample was delivered. Both practices were discontinued. After the samples had been heated and mixed until they appeared homogeneous, the milk required for one analysis was quickly poured out and weighed. The samples were weighed directly into the Mojonnier tubes at one laboratory, and at the other, they were weighed into small 25-cc. flasks and transferred to Röhrig tubes, using all of the chemicals used in the test to rinse out the flasks." Therefore it was deemed advisable to compare the different methods of transferring and of weighing samples of milk into Mojonnier extraction flasks.

PROCEDURE

The milk samples were heated to 35 to 37.5° C. in a water bath at 41.5 to 43° C., and mixed by pouring three times from one container to another. The pipettes in all three methods were filled so that the bottom of the menisci were level with the mark on the draw tubes. Each pipette delivered slightly less than 10 grams of milk at 35-37.5° C.

In method one, four pipettes were filled, the milk sample being poured once into another container between the filling of each pipette. As each

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pipette was filled, it was placed on a holder and the holder with the filled pipettes was placed on the balance pan. When the total weight was obtained, pipette number 1 was emptied into extraction flask number 1 and allowed to drain for 10 to 15 seconds. This empty pipette was again placed on the holder and the weight of milk delivered into the flask was obtained by difference. This procedure was repeated to obtain the weight of milk delivered by pipettes number 2, 3 and 4 into each of three extraction flasks that were similarly numbered. The weight of the four pipettes full of milk and the weighing stand is about 112.5 grams.

In method two, a charge of milk, using pipette number 1, was obtained from the sample, transferred immediately to a tared extraction flask, allowed to drain for 10 to 15 seconds after the flow had ceased and weighed. This was done in duplicate on each sample. The weight of the flask and charge of milk is about 76.1 grams.

In method three, a charge from the milk sample, with pipette number 1, was transferred volumetrically to an extraction flask and allowed to drain for 10 to 15 seconds after the flow had ceased. This was done in duplicate on each sample. In recording the results it is assumed that the pipette delivered 10 grams of milk.

Composite milk samples preserved with bichloride of mercury were obtained from two nearby milk plants and were sampled and transferred by methods one and two and estimated for milk fat. These plants are designated A and B. Plant A had excellent facilities for storing the composites during the bi-monthly period. They were kept constantly in a refrigerated compartment at 10° C. or lower. At the end of the bi-monthly period the samples were free of mold growth and in excellent physical condition. Plant B did not have such good facilities. The preserved milk composites were heavily contaminated with mold at the end of the bi-monthly period and the fat emulsion showed varying degrees of destabilization when tested at the milk plant. These composites were heated and prepared for testing the same as the unpreserved samples except that the cream adhering to the inside of the sample bottle was brushed loose. These preserved samples had been prepared and estimated once for milk fat by the Babcock method in the milk plant before they were brought to the Experiment Station laboratory. All the samples were selected, insofar as possible, to represent high, medium and low fat content milks in each trial.

The Mojonier (3, 4) gravimetric procedure, equipment and prescribed reagents were used to estimate the milk fat. This is a mechanized modification of the Røese-Gottlieb method. Blank determinations were made on new supplies of reagents. The ether extractions on replicate samples obtained by the three methods were made at the same time. The temperature of the laboratory and equipment did not vary greatly from 21° C., the importance of temperature having been emphasized by Mojonier (3) and Troy (6).

RESULTS

The results in table 1 show the effect of three methods of weighing and transferring 30 unpreserved and 120 preserved milk samples into extraction flasks. When milk samples are weighed in pipettes on the weighing stand (method one), there is a gradual decrease in the amount of milk fat from

TABLE 1

The effect of different methods of transferring preserved and unpreserved milk samples into extraction flasks on the estimation of milk fat by the Mojonnier method

Number of samples	Mean per cent milk fat							
	Weighed in						Measured in	
	Pipettes—Method one				Flasks—Method two	Flasks—Method three		
	1	2	3	4				
Unpreserved								
10	4.6126	4.5746	4.5260	4.5156	4.6222	4.6097	4.5849	4.5800
10	3.6572	3.6386	3.5859	3.5891	3.6949	3.6723	3.6449	3.6604
10	3.3385	3.3129	3.2897	3.2900	3.3550	3.3411	3.3225	3.3134
Preserved Plant A First Trial								
10	4.2701	4.2293	4.1541	4.1311	4.2991	4.3061		
10	3.5434	3.5395	3.4976	3.4539	3.5693	3.5680		
10	3.2142	3.1927	3.1491	3.1423	3.2332	3.2363		
Second Trial								
10	4.2718	4.2490	4.1845	4.1625	4.3031	4.2934		
10	3.5710	3.5564	3.5135	3.4994	3.6061	3.6076		
10	3.1420	3.1311	3.0821	3.0804	3.1731	3.1782		
Plant B First Trial								
10	4.5192	4.4844	4.3808	4.3876	4.6055	4.5980		
10	3.8707	3.8611	3.7870	3.7568	3.9230	3.9130		
10	3.4147	3.4008	3.3496	3.3371	3.4533	3.4482		
Second Trial								
10	4.7224	4.7059	4.6517	4.6315	4.7990	4.8054		
10	3.8834	3.8814	3.7996	3.7874	3.9421	3.9413		
10	3.5008	3.5010	3.4392	3.4348	3.5499	3.5538		

pipettes 1 to 4. The results from pipette number 1 (method one) agreed closely, but averaged slightly lower than those where the samples were weighed directly into the extraction flasks (method two). Measuring milk by volume and assuming that the pipette delivered 10 grams of milk, yielded lower results than weighing it into extraction flasks. Pipette number 1

delivered an average of 9.9402, 9.9200 and 9.9314 grams of the high, medium and low fat unpreserved milks, respectively, at 35-37.5° C. When calculations are made for the weight of the unpreserved milk delivered by this pipette, then the results obtained by measuring and by weighing milk into extraction flasks agree closely.

The results obtained by weighing the samples from Plant A into extraction flasks (method two) are slightly higher than those from pipette number 1 (method one). However, the same comparison from Plant B indicates

TABLE 2

A distribution of results from the pipettes that yielded the highest estimation of milk fat by method one on replicate samples

Milk source	Pipette number			
	1	2	3	4
Unpreserved	9	1
	9	1
	8	2
Preserved				
Plant A				
First trial	8	2
	4	5	1
	9	1
Second trial	9	1
	9	1
	10
Plant B				
First trial	9	1
	6	4
	8	2
Second trial	8	2
	6	4
	6	2	1	1
Total	118	27	2	3
Per cent of total	78.67	18.00	1.33	2.0

that significantly higher results were obtained by method two. This can be attributed to the destabilized condition of the fat emulsion in the preserved milk composites from Plant B resulting in more rapid rising of the destabilized fat in the pipettes.

The distribution of results in table 2 indicates that of the 150 estimations for milk fat that were made in replicate samples with method one, pipette number 1 yielded the highest results on 118 samples, pipette number 2 on 27 samples, pipette number 3 on 2 samples and pipette number 4 on 3 samples. The percentage distribution is 78.67, 18.00, 1.33 and 2 per cent for pipettes number 1, 2, 3 and 4, respectively. Pipette number 2 yielded the highest results on 10 unpreserved samples from Plant A and on 15 unpreserved

served samples from Plant B. It is evident that method one is not an accurate procedure to use in weighing and transferring milk samples to extraction flasks.

DISCUSSION

This study has shown that method one is not an accurate procedure for weighing and transferring samples of milk to the extraction flasks. This method is not reliable because the clusters of fat rise according to Stoke's Law and are adsorbed to the inner surface of the pipettes. The amount of cream thus adsorbed depends on the length of time that the pipettes are on the weighing stand. The amount is least in pipette number 1 and greatest in number 4. The inside diameter of the pipettes is slightly less than two centimeters. The time required to weigh and transfer four pipettes of milk (method one) to the four extraction flasks was 10.3 minutes for one technician and 12.6 minutes for the other. This would be sufficient time for some of the clusters of cream to rise in the pipettes according to the calculations by Sommer (7). In spite of the fact that four charges can be obtained with five weighings, method one is not reliable for whole milk when a high degree of accuracy is desired. The error is not great for pipettes 1 and 2, but is significant for pipettes 3 and 4. Method one was developed by Mojonnier Brothers (3, 4) especially for evaporated milk, sweetened condensed milk, ice cream, and other milk products of a homogeneous nature, but technicians might interpret the instructions to apply this method to the estimation of milk fat in whole milk. The senior author has observed the use of method one on milk in several laboratories. This method saves time because four samples can be obtained with only five weighings, while eight weighings must be made when the samples are transferred directly into the extraction flasks. The errors involved in method one would not have been considered serious twenty years ago, but now are significant with the trend toward greater accuracy and efficiency in the dairy industry. Mojonnier Brothers (5) recently emphasized that the weighing stands with pipettes are intended to be used only for products in which the fat does not readily separate.

Method two is the most reliable. It is possible to obtain a charge of milk from a well-mixed sample and deliver it immediately to the extraction flask. This is especially important in sampling milks where the fat emulsion is partially destabilized, because the destabilized fat rises rapidly in the sample jar as well as in the pipette. Therefore, it is highly important to obtain the sample quickly and deliver it immediately into the extraction flask. Mojonnier (3, 4) recommends this method for products that are not homogeneous or when the milk fat separates rapidly. When method two was used, duplicate determinations for milk fat agreed closely.

Method three may be used in commercial laboratories, provided the pipette is calibrated to deliver a definite charge of milk under standard conditions. The volumetric procedure is recommended by Mojonnier Brothers

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	1	2	3	4
Unpreserved	9	1
	9	1
	8	2
Preserved				
Plant A				
First				
trial	8	2
	4	5	1
	9	1
Second				
trial	9	1
	9	1
	10
Plant B				
First				
trial	9	1
	6	4
	8	2
Second				
trial	8	2
	6	4
	6	2	1	1
Total	118	27	2	3
Per cent of total	78.67	18.00	1.33	2.0

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Method three may be used in commercial laboratories, provided the pipette is calibrated to deliver a definite charge of milk under standard conditions. The volumetric procedure is recommended by Mojonnier Brothers

(3, 4) but they are careful to specify that their pipettes are calibrated to deliver 10 grams of milk at 15.5° C., allowing 15 seconds for the pipette to drain after the milk has ceased to flow and blowing out the last drop. A sampling temperature of 35–37.5° C., was used in this study, because it was assumed that a more representative sample would be obtained and that the pipettes would drain more completely when the milk fat is liquid. This higher sampling temperature is at least one reason why the pipettes did not deliver the specified 10 grams of milk.

CONCLUSIONS

1. Weighing milk in four pipettes on the weighing stand and transferring it to extraction flasks is not an accurate procedure. (Method one.)
2. Weighing milk directly into a tared extraction flask is the most reliable of the three methods. (Method two.)
3. Measuring milk by volume into the extraction flask with a pipette will give sufficiently accurate results for routine work provided the pipette is calibrated for the amount of milk that it will deliver under standardized conditions. (Method three.)
4. For sampling and delivering the milk sample into the extraction flask with a pipette in methods two and three, the thoroughly mixed milk should be at a temperature slightly above the melting point of the fat; for example 35–37° C.

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CHLORINE RESISTANCE OF *PSEUDOMONAS PUTREFACIENS**

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In the attempts to control outbreaks of the putrid defect in butter, chlorine solutions frequently are used to treat butter wash water and sometimes to treat plant equipment, especially churns. The difficulty in controlling the outbreaks with such procedures has suggested to certain plant operators that the causative organisms are relatively resistant to chlorine, although there remains the possibility that the suspected source of the organisms—wash water or plant equipment—was not the actual source so that the organisms were not exposed to the chlorine.

Since *Pseudomonas putrefaciens* is a common cause of the putrid defect in butter (1, 3), its chlorine resistance was investigated. Some of the cultures used were stock cultures, while others were freshly isolated for the study. The problem of chlorine resistance of *Ps. putrefaciens* is greatly complicated by the difficulty in growing the organism (2). In the studies an attempt was made to find the conditions under which it grows best.

The usual procedure was to suspend a 2-day agar culture of *Ps. putrefaciens* in sterile or pasteurized water, allow the suspension to stand for a short time at room temperature and then treat portions with the required amounts of a hypochlorite solution containing 100 ppm. chlorine. After the desired exposures, action of the chlorine was stopped by adding a solution of sodium thiosulfate. Occasionally, two concentrations of chlorine were used on the same original suspension. With each type of water some of the trials included titration of the chlorine at the beginning and end of the exposures; commonly, the chlorine concentration did not decrease significantly during an exposure and the values given represent concentrations at the ends of the exposures. The untreated and treated suspensions were cultured on special agar (2) and in some instances were used to wash butter granules obtained by churning thoroughly pasteurized sweet cream in sterile glass jars; in a few trials they also were cultured in milk.

RESULTS

Trials with sterile distilled water. Addition of 1 ppm. chlorine to a suspension of *Ps. putrefaciens* in sterile distilled water usually resulted in death of the organism in 5 seconds, but occasionally survival was noted for as long as 3 minutes; generally, suspensions requiring longer treatment contained more organisms originally than those requiring shorter treatment although other factors may have been involved. In 10 minutes the organism regularly was killed. With 5 ppm. chlorine the organism never survived a

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TABLE 1
Action of chlorine on *Ps. putrefaciens* suspended in well water

Trial	Strain of organism	Material examined	<i>Ps. putrefaciens</i> per ml.*	Butter washed with material examined†
1	1	Inoc. well water	40,000	Putrid in 2 days No change in 4 days No change in 4 days No change in 4 days
		Inoc. well water + 5 ppm. Cl for 2 min.		
		Inoc. well water + 5 ppm. Cl for 5 min.		
		Inoc. well water + 5 ppm. Cl for 10 min.		
2	1	Inoc. well water	30,000	Putrid in 2 days Slightly off in 4 days† No change in 4 days No change in 4 days No change in 4 days
		Inoc. well water + 5 ppm. Cl for 10 sec.		
		Inoc. well water + 5 ppm. Cl for 30 sec.		
		Inoc. well water + 5 ppm. Cl for 2 min.		
3	1	Inoc. well water	54,000	Putrid in 2 days No change in 4 days No change in 4 days No change in 4 days No change in 4 days
		Inoc. well water + 5 ppm. Cl for 5 sec.		
		Inoc. well water + 5 ppm. Cl for 10 sec.		
		Inoc. well water + 5 ppm. Cl for 30 sec.		
4	2	Inoc. well water	12,000	Putrid in 2 days No change in 4 days Putrid in 2 days No change in 4 days No change in 4 days
		Inoc. well water + 5 ppm. Cl for 5 sec.		
		Inoc. well water + 1 ppm. Cl for 30 sec.		
		Inoc. well water + 1 ppm. Cl for 2 min.		
5	3	Inoc. well water	16,000	Plates overgrown Plates overgrown 130 < 1
		Inoc. well water + 1 ppm. Cl for 1 min.		
		Inoc. well water + 1 ppm. Cl for 5 min.		
		Inoc. well water + 1 ppm. Cl for 10 min.		

* As determined by smears on special agar.

† Unsalted butter held at 21° C.

‡ Tests indicated that *Ps. putrefaciens* was not present.

5-second exposure, which was the shortest one used. During trials with this type of water it was noted that without chlorination *Ps. putrefaciens* remained viable for only a few hours. While survival for 1 hour, or even much less, was sufficient for conducting the tests on a suspension, it appeared that killing the organism with chlorine in this type of water would be easier than in the type of water generally used in dairy plants. Accordingly, additional tests were conducted.

Trials with pasteurized well water. Water was obtained from the college water system before filtration; after pasteurization at approximately 90° C. for 30 minutes, it was cooled to room temperature and filtered to remove the precipitated material. Table 1 gives representative results obtained with this type of water.

In trial 1 with strain 1 the original suspension of organisms had a count of 40,000 per ml. and 5 ppm. chlorine killed them with exposures of 2, 5 or 10 minutes. The unchlorinated water produced a putrid condition in unsalted butter in 2 days at 21° C. while the chlorinated samples failed to produce the defect. In trials 2 and 3, also with strain 1, satisfactory destruction of the organisms was obtained with 5 ppm. chlorine, even with very short exposures. With an exposure of only 5 seconds (trial 3) no growth was obtained on plates and no spoilage (due to *Ps. putrefaciens*) resulted when the treated water was used to wash butter. The effect of a smaller dosage of chlorine is shown in trial 4 conducted on a suspension of strain 2 containing 12,000 organisms per ml. Although 5 ppm. chlorine for 5 seconds resulted in satisfactory destruction, 1 ppm. for 30 seconds failed to kill all the organisms and unsalted butter washed with the water became putrid in 2 days at 21° C.; however, exposures of 2 or 10 minutes resulted in satisfactory destruction. In trial 5, conducted on a suspension of strain 3 containing 16,000 organisms per ml., 1 ppm. chlorine failed to give as satisfactory destruction as in trial 4 and there was some survival after 10 minutes exposure although all the organisms were killed in 20 minutes; these suspensions were not tested in experimental churnings. Presumably strain 3 was comparatively resistant to chlorine.

The data indicate that *Ps. putrefaciens* is rather susceptible to destruction by chlorine, being rapidly killed, even when present in comparatively large numbers, with a concentration of 5 ppm. With less chlorine more time is required, but again the organism displays no unusual powers of resistance. Under certain conditions, such as presence of organic matter in the water, excessive numbers of organisms or some comparable factor, less satisfactory destruction would be expected. The type of chlorine compound and the pH at which it acts need definite consideration.

Trials involving unsatisfactory destruction of Ps. putrefaciens. In certain trials chlorine failed to give satisfactory destruction of *Ps. putrefaciens*, even when allowed to act for considerable periods; the following examples illustrate this.

Example 1. A suspension of 480,000 organisms per ml. in lake water (pasteurized and filtered) was treated with sufficient chlorine to give a residual of 0.9 ppm. after 2 minutes. Under these conditions the chlorine failed to kill enough organisms in 5 seconds, 10 seconds, 1 minute or 2 minutes to show any destruction on agar plates smeared with 1 ml. or 0.1 ml. of the treated suspensions. In addition, all the chlorinated samples produced spoilage in experimental butter in the same period as the unchlorinated suspension.

Example 2. Well water (pasteurized and filtered) with a count of 620,000 organisms per ml. was treated with 1 ppm. chlorine for periods up to 2 minutes. All the exposures used failed to give appreciable decreases in the number of organisms. In another trial with well water containing 60,000 organisms per ml., 2 ppm. chlorine failed to give satisfactory destruction even after 10 minutes.

Presumably, various factors are involved in the low destruction of *Ps. putrefaciens* in certain trials. In example 1 the number of organisms was excessive for the amount of chlorine and the periods of action; in addition, the water evidently contained considerable organic matter since chlorine was rapidly dissipated in it without any addition of organisms. In the first trial of example 2 an excessive number of organisms in relation to the amount of chlorine and periods of action was again involved. However, in the second trial this apparently was not the case although the strain employed was a very recent isolation which may have grown poorly on the agar so that the count obtained was much lower than the actual number of cells present; apparently, the strain was not especially resistant because in later trials it was easily destroyed by chlorine.

Growth of Ps. putrefaciens in the test media employed. When the untreated and treated suspensions of *Ps. putrefaciens* were cultured on the surface of the special agar, growth usually was satisfactory but in a few instances suspensions failed to give the growth expected on the basis of the amount of inoculum used. This was particularly true with certain strains of *Ps. putrefaciens* and especially with some very recent isolations.

Comparisons of growth of *Ps. putrefaciens* on the special agar and in litmus milk indicated that the agar was the better medium. Often suspensions giving good growth on the agar failed to produce any change in litmus milk, even with comparatively large inoculations. There was an excellent agreement between growth of the organism on the agar and production of the putrid defect in unsalted butter at 21° C. Even when a suspension gave comparatively little growth on plates it eventually caused the defect in butter. Commonly, results were obtained more quickly on the agar, where colonies usually were evident in 24 to 30 hours, than in butter; in most cases 2 days or longer were required for production of the putrid defect in butter, even when the wash water contained many organisms.

On the basis of the results it appears that culturing on the special agar is a satisfactory method of measuring destruction of *Ps. putrefaciens* although even on this medium some cells apparently fail to grow. The agreement between growth on the agar and production of the putrid defect in butter is significant from the practical standpoint since satisfactory destruction of the organism has been attained when it does not produce a defect in butter.

DISCUSSION

Failure of *Ps. putrefaciens* to show unusual chlorine resistance would be expected because of the absence of spores and the general lack of resistance of the species of the genus *Pseudomonas* to various agents causing destruction of bacteria. When unsatisfactory destruction occurred, the cause generally was apparent and involved factors other than resistance of the organism to chlorine.

The excellent agreement between growth of *Ps. putrefaciens* on the special agar and in butter is rather surprising since it often is assumed that butter is the best medium for the organism. The comparatively poor growth in litmus milk has been noted in other investigations (4).

SUMMARY

When suspended in sterile distilled or pasteurized and filtered well water, *Ps. putrefaciens* was easily destroyed by chlorine, provided excessive numbers of cells were not present; destruction was especially active in the distilled water. When the numbers of organisms in relation to the amount of chlorine or its period of action were excessive, destruction was unsatisfactory. Among the strains tested there was some evidence of variation in chlorine resistance.

There was excellent agreement between growth of *Ps. putrefaciens* on a special agar and production of the putrid defect in unsalted butter at 21° C. Various suspensions yielding good growth on the agar failed to produce changes in litmus milk.

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OBSERVATIONS ON FISHINESS IN BUTTER*

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At one time fishiness in butter was a common and conspicuous defect. Regardless of the original quality, butter rather frequently developed a pronounced fishy flavor, especially during storage, and the financial losses involved were large. Because of its importance, the defect has been extensively investigated and the more significant factors favoring its development are now generally recognized (2, 3, 4, 5).

While at present fishiness in butter is being controlled to a large extent, the defect still is occasionally encountered. Often this fishiness is rather mild and the pronounced fishy flavors that at one time caused such serious financial losses are unusual. The mild fishiness is not generally recognized as such by the consumer, and the criticism may simply be that the butter is off in flavor.

For the most part the fishiness now encountered in butter is due to failure to apply the control procedures that have been thoroughly established. Illustrations of such failures are reported herein; all of them involve salted butter, fishiness being rare in unsalted butter.

RESULTS

Fishiness with low pH values. In all probability reduction of the acidities at which cream is churned has been the most important factor in limiting the number of fishy churnings and the intensity of the fishiness in those churnings. However, it appears that insufficient acid reduction still is of significance as a cause of the defect.

Table 1 gives the pH values of fishy and non-fishy butter from various plants; the churnings in each series were made within a very short period so that the general conditions of manufacture were much the same, but since each plant obtained cream from a relatively large area there undoubtedly were definite differences in the cream used. In each series the churning or churnings of butter having a fishy flavor had the lowest pH value or values. In some series the difference in pH between a fishy and a non-fishy churning was small.

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TABLE 1

The pH values of fishy and non-fishy lots of commercial butter from various plants
 The 2 to 6 samples from a plant were made during a short period.

Plant no.	Sample no.	Fishy flavor	pH	Plant no.	Sample no.	Fishy flavor	pH
1	1	-	6.75	5	1	-	6.76
	2	+	6.05		2	+	6.37
	3	-	6.56		3	-	6.93
	4	-	6.80		4	+	6.22
2	1	+	6.45		5	+	6.46
	2	+	6.58		6	-	7.24
	3	-	7.04	6	1	-	6.97
	4	-	6.82		2	+	6.51
3	1	-	7.32		3	-	6.91
	2	+	6.58		4	+	6.47
	3	-	7.64	7	1	-	7.02
	4	-	7.08		2	+	6.55
4	1	-	6.96		3	-	6.67
	2	-	6.78	8	1	+	6.68
	3	+	6.52		2	-	7.02
	4	+	6.64				

Distribution of the pH values of a number of fishy and non-fishy churnings of butter from each of four plants is shown in figure 1. The churnings from each plant were not necessarily consecutive but were made within a comparatively short period. In general, the fishy churnings from a plant are grouped among the churnings having relatively low pH values. Also,

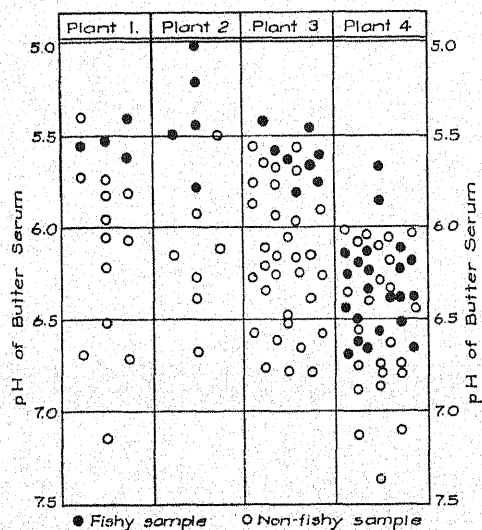


FIG. 1. Distribution of pH values of fishy and non-fishy samples of butter from four plants.

the butter from plant 4 commonly developed fishiness at higher pH values than the butter from the other three plants, suggesting that a factor other than pH also was operative.

The pH values of miscellaneous samples of fishy butter from widely scattered sources are given in table 2. In general, the values are lower than those desired in butter by various manufacturers who attempt to control the pH of their butter within rather narrow limits because of the effect of low pH on chemical deterioration in butter. Some of the values are so low that fishiness definitely would be expected to develop in the butter.

Fishiness with relatively high pH values. Occasionally lots of fishy butter have pH values which ordinarily would be considered high enough to prevent development of the defect since they definitely are above the values commonly associated with fishy butter. Because of the relationship of copper to chemical deterioration in butter, a number of such samples were

TABLE 2

The pH values of miscellaneous samples of fishy butter from widely scattered sources

Sample no.	pH	Sample no.	pH
1	5.61	11	5.35
2	5.98	12	5.95
3	5.75	13	6.15
4	5.45	14	6.14
5	6.50	15	5.98
6	6.74	16	5.88
7	6.63	17	6.44
8	6.51	18	5.68
9	6.60	19	5.35
10	5.10	20	5.80

examined for copper content, using wet ashing (6) and the carbamate procedure (1, 6). High values commonly were obtained.

The general situation is illustrated by the following case history: In a plant receiving cream from a wide area, special attention was being given to the control of pH in the finished butter, and for the most part variations in pH values of the different churnings were small. At one of the routine examinations of samples from successive churnings in the plant, the flavor scores were normal except in the case of one sample which was decidedly fishy; the samples had been held for about 3 weeks at about 7° C. so that conditions were favorable for development of the defect. It was assumed that the pH of the sample was low, but determinations indicated a value of 7.1. Copper determinations were then made and showed a content of 7.3 ppm. Other churnings made on the same day as the fishy butter contained from 0.4 to 0.75 ppm. of copper.

The plant records for the day on which the defective butter was made showed that the vat of cream used for the butter had a conspicuously lower

acidity than any of the other vats. It included a large proportion of cream from cheese factories, both that from whey and that from milk; this type of cream was reaching the plant at lower acidities than cream coming from farms, either directly or through cream stations. In various cheese factories exposed copper on equipment undoubtedly accounted for a high copper content in the whey and in the cream obtained from it.

Table 3 gives copper contents of additional samples of fishy butter having pH values so high that fishiness ordinarily would not be expected in the butter. The contents vary from 0.85 to 7.0 ppm. While data on the normal copper contents of butter in the areas involved are not available, the values given are definitely higher than those on other churnings from the same plants at approximately the same periods.

TABLE 3
Copper contents of samples of fishy butter having relatively high pH values

Sample no.	pH	Cu content <i>ppm.</i>
1	6.70	1.3
2	6.70	1.9
3	7.19	1.3
4	7.25	7.0
5	6.85	2.2
6	7.10	1.9
7	7.13	0.85

In a number of instances fishy butter was encountered which had both a high copper content and a relatively low pH value. Under such conditions the effect of the copper probably was greater than it would have been with a high pH value.

Control of the pH of butter. Adequate control of the pH of salted butter, which is so necessary under present conditions of butter manufacture, sometimes presents difficulties. Proper adjustment of the acidity of the cream at the time of churning is a common basis for such control. Under a given set of plant conditions there is a reasonably close correlation between cream acidity at churning and pH of the butter but, because of the influence of various factors on the relationship, results obtained in one plant must be tested in another plant before they can be accepted there. Also, in a plant in which the correlation has been thoroughly investigated an unusual condition may upset the relationship which has prevailed for an extended period.

In a plant which began to receive considerable volumes of cheese factory cream, both whey cream and cream from whole milk, it was noted that the butter was developing a fishy flavor soon after manufacture. Study of the butter showed that the pH values were relatively low although the churning acidities had not been changed. Eventually it was learned that certain

cheese factories were skimming a very rich cream and then diluting it with cold water to cool the cream and thin it sufficiently to make handling easier. Addition of the water changed the buffer capacity of the cream so that the pH of the cream at a given acidity was considerably lower than it would otherwise have been.

Sources of copper in butter. Equipment in certain manufacturing plants is an obvious source of copper in butter. Exposed copper in various vats, in piping, etc., contributes significant amounts of copper to cream and to the butter made from it. Presumably an alloy containing copper also can be of importance in this connection. The definite trend toward the use of special metals in the making of equipment for various types of dairy plants is a clear recognition of the objectionable effects of copper and certain other metals on dairy products.

There also are less obvious sources of copper in cream and butter, such as exposed copper in cheese plants supplying cream, unusual equipment on dairy farms, etc. Some of these may be difficult to detect because of their distance from the butter plant but should be considered when it appears that such defects as fishiness in butter are caused by excessive amounts of copper in the product.

When replacement of equipment showing exposed copper is impossible for financial or other reasons, retinning limits the copper contamination. Also, butter made from the first cream through the equipment each day should be used in channels in which it will soon be consumed rather than in channels in which consumption may be delayed. It is probable that there is a definite advantage in keeping the pH of the butter comparatively high.

Detailed studies on the normal copper content of butter are greatly needed so that it will be possible to tell when the content has been definitely increased during the collection of cream and its manufacture into butter. There is some evidence that the normal copper content of butter from various areas differs rather widely.

Distribution of fishiness between fat and serum of butter. In a number of instances the distribution of fishy flavor between fat and serum of butter was studied by melting the butter and separating the fat and the serum with a separatory funnel. The melted fat was then filtered through paper at about 45° C., and the serum was centrifuged to remove the fat as completely as possible.

The fishy flavor was very conspicuous in the fat, while there was little or no fishiness in the serum. When experienced butter judges were asked to taste the serum and identify the defect in the butter from which the serum came, they commonly could not do so; however, if the fat was not rather completely removed from the serum the fishiness was evident in the serum.

When the filtered butterfat was steam distilled the distillate was very fishy, but even after extended distillation some fishiness was still evident in

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When the filtered butterfat was steam distilled the distillate was very fishy, but even after extended distillation some fishiness was still evident in

the steamed fat. In general, the steaming had no significant effect on the acidity of the fat.

DISCUSSION

The data emphasize the importance of proper pH control in the manufacture of butter if fishiness is to be avoided and are in agreement with the fundamental studies that have been reported along this general line. They also show that fishiness still occurs in butter from various plants. Proper control of the pH of butter apparently is not as simple as it sometimes is supposed to be. This is particularly true in plants receiving cream from a variety of sources, some of which may present unusual situations from the standpoint of the relationship between the titration value on the cream and the pH of the resulting butter.

The presence of relatively large amounts of copper in butter developing fishiness when the pH values are satisfactory again is in agreement with the results of the early investigations. While contamination of cream and butter with copper in butter manufacturing plants can be controlled under normal conditions by replacement of equipment, retinning of equipment, etc., it is probable that with plants receiving cream from certain sources the cream sometimes contains excessive amounts of copper when it reaches the plants. This general situation shows the importance of a proper understanding of the conditions under which cream is handled before it comes to the butter plants.

Evidence from various sources indicates that factors other than pH and copper content influence the development of fishiness in butter. In addition to those commonly recognized, the area in which the cream is produced and the cream treatment appear to be of importance on the basis of general observations.

The cream from certain producing areas apparently yields butter which is more susceptible to the development of a fishy flavor than cream from other areas. This could easily be related to the feeds consumed by the cows because of their effect on the composition of the milk, including the content of such minor constituents as lecithin (5). However, there also are other possibilities.

Extensive treatment during the processing of cream for butter also may influence the development of fishiness. For example, certain types of pasteurization may affect the stability of the fat or fat-like constituents.

SUMMARY

Fishy butter from different plants commonly had lower pH values than non-fishy butter from the same plants at about the same periods. In various instances in which fishy butter had a relatively high pH value, it contained comparatively large amounts of copper.

Proper control of the pH of butter requires recognition of any unusual condition which develops. Certain sources of copper are very obvious but others, such as exposed copper in cheese plants supplying cream for butter manufacture, are much less obvious.

When fishy butter was separated into fat and serum, the fishy flavor was conspicuous in the fat but there was little or no fishiness in the serum.

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FINAL REPORT OF THE SUB-COMMITTEE ON THE DETERMINATION OF THE PERCENTAGE OF FAT IN SWEETENED CONDENSED MILK AND EVAPORATED MILK

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The committee has made an attempt to find a satisfactory method for the determination of fat in sweetened condensed milk and evaporated milk where Mojonnier equipment is not available.

The standard method of procedure in conducting the Babcock test is unsuitable for testing dairy products containing added sugar on account of the carbonization of the sugar which makes the reading of the fat column difficult.

Accuracy, simplicity, low cost and the use of standard Babcock equipment were the principal factors considered.

Comparisons were made of a number of methods which have been used in determining the percentage of fat in dairy products, with the Mojonnier method as the standard. Some of the methods investigated were eliminated because the originators did not recommend them for testing sweetened condensed milk and evaporated milk. Others were eliminated because the results were not satisfactory to the committee.

It was found, in making a further investigation, that both the Pennsylvania and the Minnesota methods gave results which agreed closely with the Mojonnier method. It was decided to recommend both of these methods.

PREPARATION AND AMOUNT OF SAMPLE

I. *Sweetened Condensed Milk*

To facilitate weighing the sweetened condensed milk into the test bottle, it can be mixed with an equal weight of water. This is done by balancing two beakers on opposite sides of a set of cream or similar scales; approximately 4 ounces of sweetened condensed milk are added to one of the beakers and an equal weight of water added to the other and the contents of the two beakers are thoroughly mixed together and then placed in a stoppered flask.

For both the Pennsylvania and Minnesota methods, weigh 9 grams of this mixture into either a 9-gram, 20 per cent ice cream test bottle or an 8 per

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cent milk test bottle. The results are multiplied by two if a 9-gram ice cream test bottle is used and by four if an 8 per cent milk test bottle is used.

II. *Evaporated Milk*

In the Pennsylvania method, weigh a 6-gram sample into either an 8 per cent milk test bottle or a 9-gram ice cream test bottle. Multiply the results by three when an 8 per cent milk test bottle is used and by one and one-half when a 9-gram ice cream test bottle is used.

For the Minnesota method, weigh a 9-gram sample into a 9-gram 20 per cent ice cream test bottle.

DIRECTIONS FOR USING THE PENNSYLVANIA METHOD

I. *Equipment*

Regular Babcock equipment and glassware, including ice cream test bottles, are employed.

II. *Reagents*

1. Ammonium hydroxide (28-29 per cent NH_3 is necessary).
2. Normal butyl alcohol (B.P. 117°C).
3. Diluted commercial sulphuric acid (specific gravity approximately 1.72-1.74). The acid is diluted by adding $3\frac{1}{2}$ parts, by volume, of commercial sulphuric acid, specific gravity 1.82-1.83, to one part of water, in a heat resisting container.

III. *Procedure*

The same procedure is followed in testing the sweetened condensed milk and evaporated milk even though the weight of sample varies. The procedure is as follows:

1. Add 2 milliliters of ammonium hydroxide from a burette.
2. Mix for approximately one-half minute.
3. Add 3 milliliters of butyl alcohol from a burette.
4. Mix for approximately one minute.
5. Add 17.5 milliliters of the diluted sulphuric acid.
6. Mix thoroughly until digestion is completed.
7. Centrifuge the bottles for 5 minutes.
8. Add water ($130-140^\circ \text{F}$.) to bring the contents to within one-fourth inch of the base of the neck of the bottle.
9. Centrifuge for two minutes.
10. Add enough water ($130-140^\circ \text{F}$.) to keep the fat within the graduated portion of the neck of the bottle until read.
11. Centrifuge one minute.
12. Place the bottles in a water bath at 130°F .
13. Allow a few drops of glymol to run down the inside of the neck of the bottle just before reading. Measure the length of the fat column from the

bottom of the lower meniscus to the sharp line of demarcation between the glymol and the fat.

DIRECTIONS FOR USING THE MINNESOTA METHOD

I. *Equipment*

Regular Babcock equipment and glassware, including ice cream test bottles, are employed.

II. *Reagents*

Minnesota Babcock reagent.

III. *Procedure*

The same procedure is followed in testing the sweetened condensed milk and evaporated milk, even though the weight of sample varies. The procedure is as follows as recommended by Thurston and Brown:*

1. Add 15 milliliters of Minnesota reagent.
2. Shake thoroughly.
3. Digest 12 to 15 minutes in a gently boiling water bath, having the bottles in a rack held at least 2 inches above the bottom of the bath.
4. Shake the mixture in the test bottle vigorously at the time when at least half the contents of the bottle have turned dark brown (usually about 2½ minutes after placing them in the water bath).
5. Shake vigorously again about one minute later. (Note: Some care may be necessary when starting to shake the bottles the second time, as the alcohol in the reagent may boil off through the neck of the bottle, taking with it some of the mixture.)
6. Place the tests in a centrifuge and centrifuge them for one-half minute at the speed used for the Babcock test.
7. Add hot water (130–140° F.) to float the butterfat well up into the neck of the test bottle.
8. Centrifuge for one-half minute.
9. Place the tests in a water bath at 133–137° F. and leave for 5 minutes.
10. Just before reading each test, allow colored reading fluid (such as glymol) to flow gently onto the surface of the fat column.
11. Hold the bottles in a level position and read as one would read a Babcock cream test.

REMARKS

Either method can be expected to give reliable results within the accuracy of reading the calibrations on the Babcock test bottle and both are well adapted to the determination of fat in sweetened condensed milk and evaporated milk.

* Ice Cream Field, February 1937.

THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR PRODUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE.

II. VITAMIN A *PER SE**

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In previous experiments (8) it was found that the vitamin A requirement of dairy cows for the production of milk fat with maximum vitamin A value was approximately 550,000 units per day, when the source of vitamin A in the ration was artificially dried alfalfa hay (carotene). Recent experiments have been completed to determine this daily requirement when the source of vitamin A in the ration was vitamin A *per se* (fish liver oil).

EXPERIMENTAL

The general plan of procedure in all of these experiments has been to decrease the vitamin A potency of the milk secreted by the cows to a low level by feeding vitamin A deficient rations and then determine the number of vitamin A units required daily by the cows to restore the vitamin potency of the milk fat to a high level.

The vitamin A deficient ration was composed of beet pulp, and a grain mixture consisting of white corn, oats, linseed oil meal, bone meal and salt. The source of vitamin A in the repletion rations was vitamin A *per se* (fish liver oil).

Throughout this report vitamin A activity is quantitatively expressed as Sherman-Munsell units.

Experiment I

Two Guernsey cows in the early stage of lactation were used in this trial. The cows were fed the vitamin A deficient ration until the vitamin A potency of their milk fat dropped to a low level (12 units per gram). Beginning at this point each cow was fed vitamin A doses of 25,000, 50,000, 75,000, 100,000, and 200,000 units daily in successive 21 day feeding periods.

Representative samples of milk were collected from each cow during the last two days of each period from which butter samples were prepared for biological assay. Each sample of butter was assayed separately for vitamin A potency.

The results of experiment I are shown in table 1 and figure 1.

Experiment II

Two Guernsey cows were used in the feeding trials of this experiment. After their stores of vitamin A had been depleted to a low level, one cow

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TABLE 1

Showing the vitamin A requirement of dairy cows when vitamin A *per se* was the source of vitamin A in the ration

Trial 1

Period No.	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.	
		Cow 521	Cow 535
1	Depletion	12	12
2	25,000	10	12
3	50,000	11	12
4	75,000	15	17
5	100,000	30	30
6	200,000	36	34

* Sherman-Munsell units.

was fed daily vitamin A *per se* in units ranging from 50,000 up to 300,000 and back to 50,000 in successive 21 day feeding periods. The other cow, at the end of depletion period, was started on 300,000 vitamin units daily with descending and then ascending quantities fed in successive feeding periods. The feeding schedule of the two cows is shown in table 2.

DISCUSSION

As has been stated previously (8) the criterion for the measurement of the vitamin A requirements of dairy cows for the secretion of milk fat with maximum vitamin A value is based upon the supposition that cows are not able to secrete butterfat of maximum vitamin A value until the optimum requirements for maintenance and production have been satisfied. Therefore, the minimum vitamin A intake which will produce the maximum effect upon the milk fat secreted should be the minimum vitamin A requirement of the cow for the production of milk fat of maximum vitamin A value.

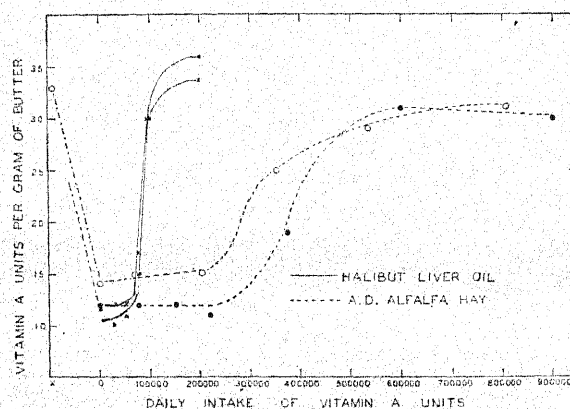


FIG. 1. Showing the relative efficiency of vitamin A *per se* and carotene (artificially dried alfalfa hay) for the production of butter of maximum vitamin A value.

TABLE 2

Showing the vitamin A requirements of dairy cows when vitamin A *per se* was the source of vitamin A in the ration

Period No.	Trial 2—Cow 534		Trial 3—Cow 535	
	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.	Daily vitamin A unit* intake	Vitamin A in butter units*/gm.
1	Depletion	12	Depletion	15
2	50,000	15	300,000	33
3	75,000	18	200,000	33
4	100,000	24	150,000	30
5	150,000	30	100,000	28
6	200,000	32	75,000	21
7	300,000	35	50,000	14
8	200,000	34	0	14
9	75,000	27	100,000	27
10	0	16	200,000	31

* Sherman-Munsell units.

Previous tests have shown that when dehydrated alfalfa hay was used as a source of vitamin A (carotene) the cows required approximately 550,000 vitamin A units daily to restore the vitamin A potency of the butterfat to its highest level.

In the present tests when the source of vitamin A in the ration was vitamin A *per se* (fish liver oil), these requirements were satisfied with approximately 200,000 units daily. For simplicity in showing the relative efficiency of these two sources of vitamin A, the daily unit intake required of each is shown in figure 1. Data for the carotene (dehydrated alfalfa hay) requirements are taken from previous experiments (8).

In trial I, it became evident that the vitamin A requirement for the production of milk of high vitamin A value was between 150,000 and 200,000 units daily when the source of vitamin A in the ration was vitamin A *per se*. In order to check these results and to determine the requirements as affected by depletion and repletion of body storage trials 2 and 3 were conducted.

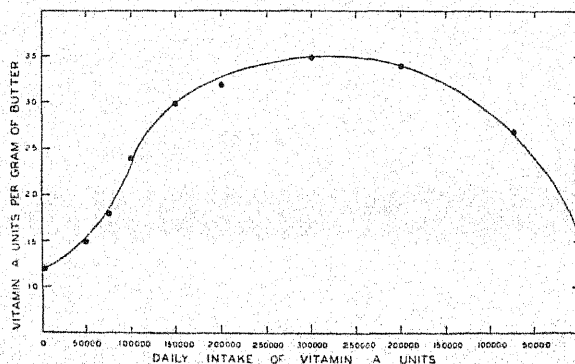


Fig. 2. Showing the vitamin A *per se* requirements for the production of butter with maximum vitamin A value.

As shown in table 2 and figures 2 and 3 the results of the second experiment were almost identical with those of the first experiment. As can be seen in figure 2 when the vitamin A *per se* in the ration was increased from 50,000 to 100,000 units daily there was a significant increase in the vitamin A potency of the milk fat secreted by the cows. The vitamin A value of the milk fat reached its maximum value when the ration contained approximately 200,000 units daily. Increasing the daily vitamin A intake to 300,000 units failed to produce any significant increase in the amount of vitamin A in the milk fat. On the other hand when the amount of vitamin A in the ration was decreased below 200,000 units daily in successive 21-day periods there was a corresponding decrease in the vitamin A potency of the milk fat secreted by the cows. There was, however, a slight lag during the depletion which was probably due to the buffering action of reserve body stores which had accumulated on the high levels.

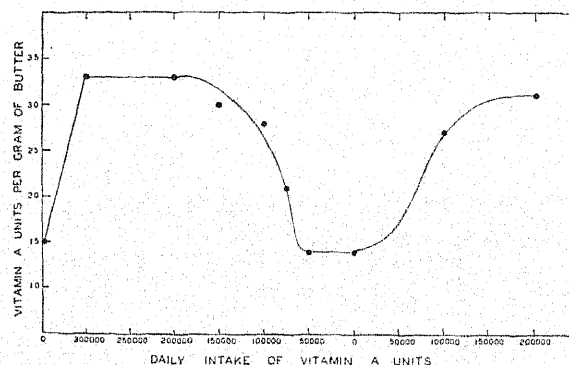


FIG. 3. Showing the vitamin A *per se* requirements for the production of butter with maximum vitamin A value when measured by depletion and repletion tests.

When 300,000 units were fed in the first period following a short depletion period the vitamin A potency of the milk fat was restored to its maximum value immediately. This is shown in table 2 and figure 3. Decreasing the unit intake to 200,000 daily failed to have any significant effect on the vitamin A value of the milk fat. With each increment decrease below 200,000 units daily there was a decrease in the vitamin A potency of the milk fat.

Following the period in which the daily unit intake had been reduced to 50,000 units the cow was again placed on the vitamin A deficient ration for 21 days, during which time the vitamin A potency of the milk fat dropped to a low level (14 units). Following this depletion period 100,000 and 200,000 units was again included in the ration in two successive 21-day periods.

As can be seen in table 2 and figure 3 there was a slight lag in the restoration of the vitamin A potency of the milk fat following this depletion period.

This was probably due to the use of a portion of the vitamin A intake for repletion of body tissues. It should be noted, however, that when 200,000 units were fed daily the vitamin A value of the milk fat again approached its maximum value.

It has been generally recognized that there exists a threshold level for the secretion of vitamin A in milk fat above which more vitamin A or carotene in the feed does not affect the vitamin A potency of butterfat (1, 2, 6, 7). On the other hand, Denel *et al.* (3) failed to show a definite threshold level in their experiments when they reported that the feeding of shark liver oil produced butterfats of exceptionally high vitamin A potencies as determined by colorimetric and spectrophotometric methods. Rusoff *et al.* (7) reinvestigated this problem and found that feeding 2,144,000 units daily failed to raise the vitamin A potency of the milk above the characteristic threshold level when the vitamin A potency was measured by biological assays. Jensen *et al.* (5), while studying the effect of feeding shark liver oil, found a threshold level in the vitamin A content of blood plasma. The results of our experiments indicate that a definite threshold level for vitamin A does exist. Under the conditions of these experiments, a daily intake of approximately 200,000 units of vitamin A *per se* or 550,000 units of carotene are required to reach this threshold level.

The minimum vitamin A requirement of dairy cows for the secretion of butterfat of maximum vitamin A value has been found to be approximately 200,000 units daily when the source of vitamin A in the diet is vitamin A *per se* and 550,000 units when supplied by carotene in dehydrated alfalfa hay. This would indicate that on the basis of daily unit intake, carotene is only about one-third as effective as vitamin A. Similar differences in effectiveness of these two sources of vitamin A have been reported by Guilbert *et al.* (4) when they studied the minimum carotene and vitamin A requirements of cattle, sheep and swine for the prevention of night blindness.

Although there appears to be a difference in the vitamin A requirements of the dairy cow when vitamin A *per se* or carotene are fed, it is conceivable that the actual physiological requirement is the same. The difference in effectiveness may be due to lower metabolic efficiency in the utilization of carotene or possibly to relative unavailability of the carotene in plant tissue.

SUMMARY

1. Feeding experiments have been completed to determine the minimum vitamin A requirements of dairy cows for the production of butter of maximum vitamin A value.
2. When the source of vitamin A in the ration was vitamin A *per se* (fish liver oil), this requirement was satisfied with a daily intake of 200,000 units.
3. In this series of experiments, vitamin A *per se* was found to be approximately three times as effective as carotene in dehydrated alfalfa hay.

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THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR PRO-
DUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE.
III. AVAILABILITY OF CAROTENE IN DEHYDRATED
ALFALFA HAY AS COMPARED WITH
CAROTENE IN OIL¹

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In previous reports (4, 9), it has been shown that the apparent vitamin A requirement of dairy cows for the secretion of milk fat with maximum vitamin A value depends upon the source of the vitamin A in the ration. When the source of vitamin A in the ration was carotene from dehydrated alfalfa hay, it was found that the daily requirement was approximately 550,000 Sherman units (9). With vitamin A *per se* this requirement was satisfied with a daily intake of approximately 200,000 units per day (4). Similar differences in the relative efficiency of utilization of carotene and vitamin A by cattle, sheep and swine have been reported by Guilbert *et al.* (2).

Carotene in some plant tissues has been found to be less effective biologically than would be indicated by analyses. Wide variations in the efficiency of utilization of carotene from different plant sources have been reported by Smith and Otis (8) and by Graves (1).

The purpose of this investigation was to compare the availability and efficiency of utilization of crystalline carotene with carotene in dehydrated alfalfa hay for the production of milk fat of high vitamin A value.

EXPERIMENTAL

Two groups of two Guernsey cows each were used in the feeding trials to determine the relative availability of the carotene in dehydrated alfalfa hay as compared with crystalline carotene in oil. The cows were similar in respect to stage of lactation, milk production and body weights. The body stores of vitamin A in the cows were depleted in a preliminary feeding period by feeding a vitamin A deficient ration consisting of beet pulp and a grain mixture of 400 lbs. white corn, 200 lbs. oats, 150 lbs. linseed oil meal, bone meal and salt. In successive feeding periods, the carotene of the hay and the carotene in the oil were equilibrated at levels of 130, 200, 300, and 200 milligrams daily during the respective feeding periods of 21 days each.

The dehydrated alfalfa hay was sacked immediately after dehydration and stored. Prior to each feeding period, sufficient number of sacks of hay

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for the next feeding period were set aside, carefully sampled, and analyzed for carotene. In preliminary determinations of carotene it was observed that the Peterson-Hughes method (6) gave consistently higher values than the methods of Hegsted, Porter and Peterson (3) and Moore (5). Since in each of these procedures a different principle is involved for the removal of the non-carotene chromogens, tests were made to determine the effectiveness of each procedure. Samples of dehydrated alfalfa hay were extracted according to the Peterson-Hughes procedure. The final petroleum ether extracts were washed with water and made to volume. Critical studies upon aliquots from these solutions disclosed that extraction of the non-carotene pigments with 94 per cent diacetone alcohol or by adsorption upon a dicalcium phosphate column were equally effective and gave carotene values in close agreement, while extraction with 90 per cent methanol failed to remove

TABLE 1
A comparison of the relative availability of carotene in dehydrated alfalfa hay and crystalline carotene in oil

Period	Group No.	Carotene supplement*		Butterfat	
		Dehydrated alfalfa hay	Carotene (in oil)	Sample No.	Vitamin A potency
		<i>mg. per day</i>	<i>mg. per day</i>		<i>Sherman units per gram</i>
1	1	None	None	1	11
	2	None	None	2	13
2	1	130	None	3	19
	2	None	130	4	19
3	1	200	None	5	32
	2	None	200	6	34
4	1	300	None	7	37
	2	None	300	8	36
5	1	None	None	9	22
	2	None	None	10	21
6	1	None	200	11	32
	2	200	None	12	33

* Ration consisted of beet pulp and a grain ration consisting of 400 lbs. white corn, 200 lbs. oats and 150 lbs. linseed oil meal.

some of the pigments and gave high carotene values. When these latter solutions were purified by further treatment with either diacetone or dicalcium phosphate, the carotene values were lowered to agree with the others. Therefore, in these experiments, the carotene values obtained by the Moore procedure (5) were used in calculating the amounts of hay to be fed daily to the cows.

The crystalline carotene in oil was purchased from the National Research Associates, Inc., as a product known as "Research" Carrot Oil which had been prepared from carrots as a thick suspension of crystalline carotene in oil (approximately 3.2 per cent carotene). Upon receipt of the oil, it was removed from the container, thoroughly mixed and bottled in 250-ml. brown bottles under an atmosphere of nitrogen. Samples were taken from a num-

ber of bottles for carotene analyses. These gave concordant results, indicating that the distribution of carotene was uniform. To prevent deterioration, the carrot oil was kept in cold storage until used.

For the carotene analyses, aliquots were dissolved in petroleum ether and the carotene determined with a Bausch and Lomb Spectrophotometer. By passing the petroleum ether solution through a dicalcium phosphate column to remove the non-carotene pigments, it was found to contain 5 per cent impurities. According to the chromatographic analyses which were made by J. W. White, Jr., the carotene was composed of 25.2 per cent alpha-carotene and 74.8 per cent beta-carotene.

Since alpha-carotene possesses but half the biological activity of beta-carotene, it was necessary to equilibrate the carotene of the carrot oil to a biological equivalence of beta-carotene. On the basis of these corrected carotene values, the requisite amount of carrot oil for each feeding dose was weighed into No. 10 gelatin capsules and fed to the cows.

At the end of each feeding period 24-hour composite samples of milk from each group were collected and the cream separated and churned into butter. The butter samples were stored at -18° C. and the portions were removed as needed for biological assays. The butterfat was heated to 55° C. and filtered to remove curd and water. The results of the biological assays of the butterfats are given in table 1.

DISCUSSION

In previous experiments (4, 9), it was found that the carotene of dehydrated alfalfa hay was only about one-third as efficient as vitamin A *per se* as a source of vitamin A in the rations of dairy cows for the production of milk fat of maximum vitamin A value. This difference might be due either to the relative unavailability of carotene in the plant tissue, or if available, to differences in the metabolic efficiencies. A comparison of the availability of crystalline carotene and the carotene in plant tissue should give an insight into this problem.

To test these possibilities, it was imperative that comparisons be made at levels which would be potentially capable of producing differences in the vitamin A potencies of the butterfats. If comparisons were made at too low levels, it is conceivable that differences might exist which would not be reflected in the vitamin A potencies of the butterfat. Likewise, if comparisons were made at levels above the minimum requirements of the cow for the production of butterfat of maximum vitamin A value differences would not be measurable. Therefore, levels of carotene intake were selected which might be expected to be intermediate between these two points.

The results of these experiments, as shown in table 1, indicate that the dairy cow can utilize the carotene in alfalfa hay as readily as isolated carotene for the production of butterfat of high vitamin A value. It is interesting to note that the vitamin A potencies of the butterfats produced by the

two groups on each of the different levels were very similar. This is true even when the two groups were reversed in period 6.

In experiments to determine the minimum carotene requirements of cattle, swine, and sheep for the prevention of night blindness, Guilbert *et al.* (2) found that the requirements were practically the same when the carotene was furnished by alfalfa or by crystalline carotene dissolved in cottonseed oil. In experiments to determine the requirements of chickens for vitamin A when fed as carotene, Sherwood and Fraps (7) found that there was no apparent difference in the effectiveness of carotene supplied in alfalfa meal or by crystalline carotene in oil.

Considering the evidence from all these experiments, it is apparent that the difference in effectiveness of carotene in alfalfa hay and vitamin A *per se* is not due to the unavailability of the carotene in the plant tissue but rather is due to differences in the metabolic efficiencies.

SUMMARY

1. Experiments have been conducted to determine the relative availability of carotene in dehydrated alfalfa hay as compared with isolated carotene (in oil) as a source of vitamin A in the rations of dairy cows.

2. Two groups of cows were used in these experiments. Each group of cows was fed equal amounts of carotene at levels of 130, 200, and 300 milligrams daily.

3. The results of these experiments indicate that dairy cows can utilize the carotene from the alfalfa hay as readily as isolated carotene.

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TEMPERATURE ERRORS IN WEIGHING AND THEIR CONTROL IN THE MOJONNIER FAT TEST

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The Mojonnier test for fat is one of the most accurate methods available for the analysis of dairy products. Nevertheless, analyses of duplicate samples made in different laboratories sometimes differ widely. It is the purpose of this paper to present data concerning one source of error in this test, temperature variations; and to describe an improved method of temperature control for Mojonnier machines.

EXPERIMENTAL

Experiment 1

It is a common observation that the weight of Mojonnier fat dishes can be reduced by warming. This is easily explained. When the dish is warmed, the air in it expands, and a part of it is expelled from the dish as effectively as if a cover had been placed over it and a part of the air had been removed with a vacuum pump. As a result, the dish will appear lighter in weight. In order to avoid such errors, the temperature of the air inside the dish must be the same as that of the air surrounding the dish.

Quantitative data on the relation between temperature and weight were obtained by weighing dishes first at one temperature and then at another. This was accomplished by placing two Mojonnier machines side by side and adjusting the water in one reservoir so that it was about 3° C. above the temperature of the water in the other machine. Four clean fat dishes were placed in the cooling oven of the warmer machine and, after 10 minutes, each dish was weighed. Immediately after weighing, the dishes were placed in the cooler machine. When all of the dishes had been weighed from the warmer machine, they were re-weighed from the cooler machine. This procedure of weighing the dishes first from one machine and then the other was repeated several times. The data are shown in figure 1. The upper line indicates the successive temperatures at which the dishes were equilibrated. The lower line shows the average weight of the four dishes after equilibrating at the temperature shown above. It is evident that a variation of only one degree in temperature may cause a change of approximately $\frac{3}{4}$ mg. in weight. This is equivalent to an error of .07 per cent of fat when testing heavy cream.

Experiment 2

It seemed desirable to find a method of reducing errors caused by improper temperature of the cooling oven. Two procedures were tried. First,

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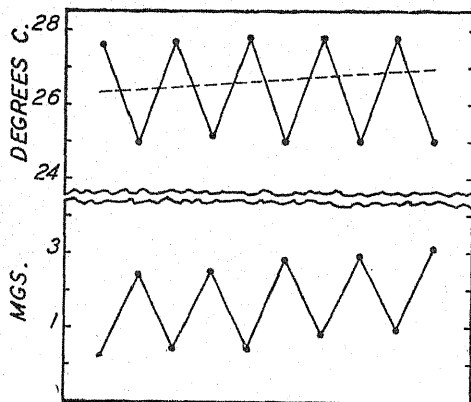


FIG. 1. Variation in the weight of Mojonnier fat dishes (below) due to variation in temperature of the dishes (above). (Actual average weight, 36.0600 grams plus the value shown.)

experiment No. 1 was repeated except that each dish was waved slowly through the air for fifteen seconds immediately before it was weighed. Second, the dishes were removed from the cooling oven and, before weighing, they were placed for two minutes upon a large sheet of $\frac{1}{8}$ -inch thick aluminum. This sheet was supported only at the corners so that air could circulate around it freely.

The data are presented in figures 2 and 3. It is evident that both procedures reduced the error due to improper machine temperature, but even the second procedure was not entirely adequate.

Experiment 3

An examination of figure 1 shows that there was a constant difference in weight of about 2 mg., depending upon whether the dishes were weighed

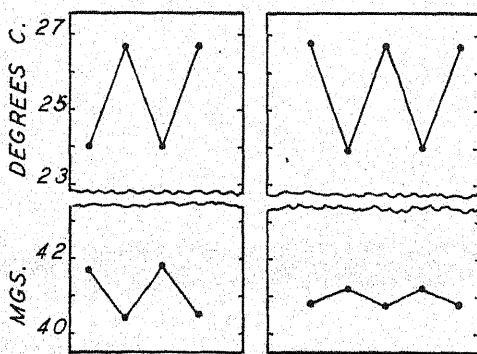


FIG. 2.

FIG. 3.

Variation in the weight of Mojonnier fat dishes (below) after tempering in oven at the temperature indicated above. Figure 2, dishes waved in air fifteen seconds before weighing. Figure 3, dishes left on metal plate for two minutes before weighing.

from the cold or the warm machine. It is evident, also, that all the dishes increased in weight in successive weighings at the same temperature. This slow increase in weight was due to a change in the temperature of the laboratory. The actual change in temperature is recorded by the broken line in the upper part of figure 1. It must be remembered that an error in weight may occur if either the temperature of the room, or the temperature of the dishes, is changed.

The following procedure was used to measure the effect of changes in room temperature upon the weight of fat dishes. A set of four dishes was placed in a Mojonnier machine. The water in this machine was held at a constant temperature while the room was warmed slowly by steam radiators. Three electric fans were used to distribute the heat uniformly through all parts of the laboratory. At intervals, the dishes were removed from the machine, weighed at once, and then returned to the cooling oven. The broken line in figure 4 shows the relation between the weight of the dishes

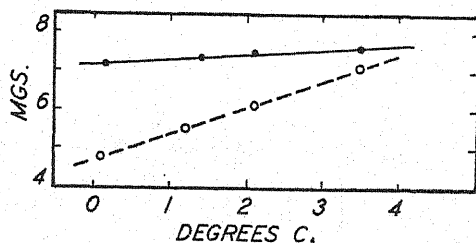


FIG. 4. The relation between the weight of Mojonnier fat dishes and increases in the laboratory temperature. (Machine temperature held constant.) Broken line: weighed direct from cooling oven. Solid line: held on metal plate for two minutes before weighing.

and the increase in the temperature of the room. The water temperature remained constant within 0.1°C .

This experiment was repeated with one change in procedure. Before weighing, each dish was placed on the large aluminum sheet for a period of two minutes. These data are shown by the solid line in figure 4.

Experiment 4

The Mojonnier machine is designed to bring all dishes to the same temperature before they are weighed. Unfortunately, in most cases, this is not room temperature. In those laboratories where the temperature varies considerably during a 24-hour period, this may lead to appreciable errors. If error is to be avoided, the dishes should be brought to the temperature of the balance, regardless of what that temperature may be, before the dishes are weighed. To accomplish this, some means should be provided for maintaining the cooling oven always at room temperature. It would seem much wiser to circulate the cooling water through an air-cooled radiator than to use a large water reservoir in a Mojonnier machine.

This idea was tested by substituting a small radiator from an automobile heater for the water reservoir of one machine. Air was blown over the radiator by an electric fan. A standard Mojonnier machine was used as a control in comparison with this air-cooled machine.

Two sets of clean fat dishes were used, one set for each machine. The experiment was divided into four periods:

1. All dishes were left on top of the balance case until they showed constant weight. This may be considered the true weight of the dishes, free from temperature errors.

2. Each set of dishes was weighed several times from its own cooling oven to get an average value which was reproducible. Since the cooling ovens had not been adjusted to exactly room temperature, these weights differed from the true weights.

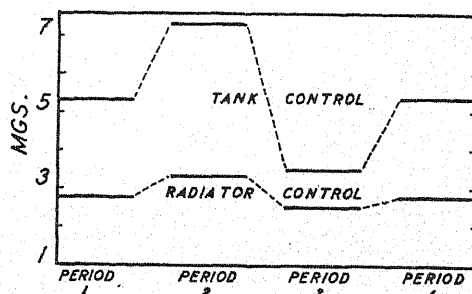


FIG. 5. A comparison of the ability of a tank-controlled machine and a radiator-controlled machine to maintain dishes at constant weight when the room temperature varies. Period 1, dishes at room temperature. Period 2, dishes weighed from cooling oven. Period 3, laboratory cooled 5° C., dishes again weighed from oven. Period 4, dishes at room temperature (5° C. lower than during period 1).

3. The laboratory was cooled 5° C. during a period of 90 minutes. Four electric fans were used to maintain uniform temperature distribution throughout the laboratory. When the room had been cooled, the dishes were weighed again from their respective ovens.

4. The fourth and final set of weighings was made after the dishes had rested upon the top of the balance case long enough to come to the temperature of the room. This set of weighings is comparable with the first except that the laboratory had been cooled 5° C.

The data are presented in figure 5. It is evident that the use of an air-cooled radiator in place of a water reservoir on a Mojonnier machine would be quite helpful in any laboratory which is subject to temperature variations.

Experiment 5

As a further test of the ability of the small radiator to maintain the cooling oven at room temperature, the room was cooled very rapidly, and

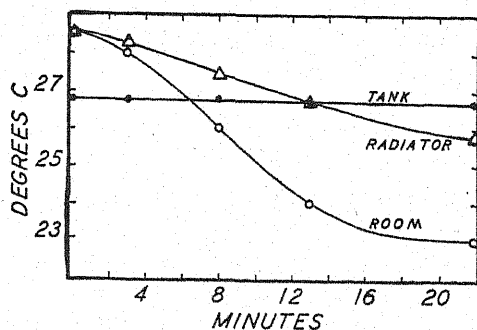


FIG. 6. The comparative rates of temperature adjustment of a tank-controlled machine and a radiator-controlled machine during a period of rapid change in laboratory temperature.

records were kept of the temperature of the room, the temperature of the water circulating through the cooling oven of a standard Mojonnier machine, and the temperature of that circulated through the oven of the air-cooled machine.

The data are shown in figure 6. It is evident that the radiator was too small to keep up with such rapid changes in room temperature though, in this respect, it was far superior to the reservoir on the other machine.

Experiment 6

The magnitude of the error due to improper temperature of the dishes is dependent upon the speed with which they are weighed. If the operator were slow enough, the dishes could come to room temperature while he was adjusting weights. In all of the experiments reported thus far, the actual weighing was performed as rapidly as possible, but no time records were kept.

An experiment was carried out to determine the effect of speed of weighing upon the errors in weight. Dishes were removed from an oven which

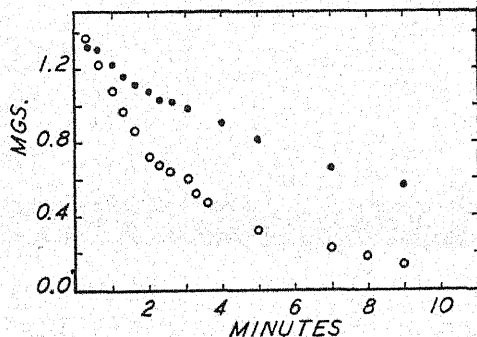


FIG. 7. The rate of cooling of two Mojonnier fat dishes in the balance case as measured by differences between the weight observed at various intervals after removing from the Mojonnier machine and the final equilibrium weight.

was slightly warmer than the room and placed at once upon a balance. Apparent weights were recorded at intervals for thirty minutes, or until the weight remained constant. The final weight was considered the true one, and the errors of earlier weighings were calculated accordingly. Such measurements indicate how slowly the dishes acquire the temperature of the balance case. It was found that different dishes cooled at different rates. Typical data are shown in figure 7. To permit the use of a larger scale in plotting, data are given for only the first ten minutes of the cooling period.

SUMMARY

Errors in Mojonnier fat tests may occur whenever the temperature of the room varies during the course of the analysis. Data are presented showing the magnitude of such errors.

It would be preferable to circulate the water for a Mojonnier machine through an air-cooled radiator instead of through a reservoir. Data are given showing the advantages of such a device.

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Thirty-Ninth Annual Meeting, Ohio State University, Columbus, Ohio.

June 20, 21, 22, 1944

FIRST CALL FOR TITLES

Titles of original papers to be presented should be in the hands of the Program Committee not later than April 1, 1944. All communications regarding general program plans should be addressed to Professor H. P. Davis, General Program Chairman, Dept. Dairy Husbandry, University of Nebraska, Lincoln, Nebraska. The personnel of the sectional program committees are listed in the preceding list.



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ACTION OF BUTTER CULTURES IN BUTTER: A REVIEW*

F. J. BABEL AND B. W. HAMMER

Iowa Agricultural Experiment Station, Ames, Iowa

CONTENTS

Introduction	80	Influence of addition of citric acid to cream	113
Methods of using butter cultures in the manufacture of butter	80	Influence of aeration of cream	114
Salted butter	80	Influence of neutralization and pasteurization of cream	116
Adding butter culture to pasteurized cream and holding the mixture cold for some hours	81	Influence of certain metallic salts	117
Adding butter culture to pasteurized cream and ripening somewhat	82	Influence of churning	117
Adding butter culture to pasteurized cream at churning	84	Influence of washing butter with water	117
Adding butter culture directly to butter	86	Partition of acetylmethylcarbinol and diacetyl during churning	118
Unsalted butter	87	Comparative amounts of acetylmethylcarbinol and diacetyl in cream and butter	118
Use of special cultures in butter manufacture	88	Comparative amounts of acetylmethylcarbinol and diacetyl in cream and butter-milk	120
Survival and growth of butter culture organisms in butter	90	Comparative amounts of acetylmethylcarbinol and diacetyl in butter and butter-milk	121
Salted butter	90	Partition of acetylmethylcarbinol and diacetyl between aqueous and fatty constituents of cream and butter	122
Unsalted butter	92	Influence of reworking butter	125
Sources of natural butter flavor	93	Influence of storage of butter	125
Effect of butter culture on flavor of butter	94	Salted butter	125
Effect of butter culture on keeping qualities of butter	95	Unsalted butter	128
On general score	95	Action of acetylmethylcarbinol and diacetyl on butter	129
In preventing certain bacterial defects	99	Chemical considerations	130
Relationship of specific chemical compounds to desirable flavor of butter	101	Microbiological considerations	132
Volatile acids	102	Amounts of acetylmethylcarbinol and diacetyl in various dairy products	132
Acetylmethylcarbinol and diacetyl	103	Milk	133
Homologs of diacetyl	105	Skimmilk	133
Miscellaneous	105	Cream	133
Factors influencing acetylmethylcarbinol and diacetyl contents of butter	106	Other products	134
Influence of strain of butter culture	106	Butter	134
Influence of acidity of cream	107	Butter culture distillate as a source of flavor for butter	136
Influence of temperature of ripening	110	Detection of added diacetyl in butter	137
Influence of fat content of cream	112		

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INTRODUCTION

The butter industry early recognized that the type of souring in cream largely determines the flavor of butter freshly prepared from it, and attempts were made to control the souring of raw cream intended for churning by addition of clean-flavored sour milk or cream, buttermilk from a churning of good butter or some similar material. Although pasteurization of cream for butter originally was intended primarily to improve the keeping qualities of the butter, it also permitted better control of the fermentation of the cream through use of selected cultures of bacteria in a medium relatively free of other micro-organisms. Pasteurization of cream and flavor development through use of selected cultures of bacteria increased simultaneously in the butter industry during the final decade of the last century, each tending to complement the other under the general system of butter manufacture then followed. With the recognition of the relationship of high churning acidities in cream to chemical deterioration in butter, use of low acid cream, either sweet or neutralized, and changes in the methods of employing butter cultures were indicated and soon were widely followed.

The present review deals with the general action of butter cultures in butter. It supplements a review (43) dealing with the bacteriology of butter cultures, including the chemical changes brought about in milk through activity of the normal butter culture species.

METHODS OF USING BUTTER CULTURES IN THE MANUFACTURE OF BUTTER

When butter cultures first came into general use, the common practice was to ripen the cream, either raw or pasteurized, to a comparatively high acidity and then cool and churn. On holding, butter made in this manner sometimes developed serious flavor defects because of the influence of acid on chemical changes in it and, accordingly, methods of employing cultures were developed which involved churning the cream at a relatively low acidity.

The method of using cultures in a plant often is determined largely by the type of butter desired, as well as by the plant equipment and plan of operation. Salted butter for immediate consumption can be churned at a higher acidity, so that it will have more flavor, than butter to be stored for considerable periods during which serious chemical deterioration may occur. With unsalted butter, a high churning acidity ordinarily is employed because in the absence of added salt a high acidity is relatively unimportant as a cause of deterioration; moreover, the flavor desired in unsalted butter intended for table use is best obtained through a high churning acidity.

Salted Butter

At present the more common methods of using butter cultures in the making of salted butter are:¹

¹ A report involving a comparison of several methods of using butter culture is reviewed under the method which gave the best results.

Adding butter culture to pasteurized cream and holding the mixture cold for some hours. An important method of using butter culture is to add 2 to 8 or more per cent to pasteurized and cooled cream, hold the mixture cold for some hours, preferably overnight, and churn. While the added culture raises the acidity of the cream somewhat, there is no significant increase in acid during the holding because the low temperature largely prevents the action of the culture organisms.

Wilster (138) noted the most improvement in the flavor of butter when the cream, with culture added, was held 16 hours below 40° F.,² or when the cream was ripened to 0.28 per cent acid (0.42% in the serum) and held overnight below 40° F. With these methods the average scores of the butter were increased nearly 1 point compared to butter made without culture or to butter made with culture added to the cream immediately before churning.

In 28 comparisons, Fabricius and Hammer (33) found addition of 8 per cent culture to sweet or slightly sour cream 16 hours before churning gave butter significantly more often high in score than addition just before churning; this was the case with both the fresh and the stored butter. In 11 trials in which acetylmethylcarbinol plus diacetyl³ determinations were made on the cream at churning, addition of culture 16 hours before churning commonly resulted in higher values than addition at churning. This was not the case in two of the trials and with these the butter made by adding culture 16 hours before churning scored the lower. In another series of experiments, no culture was added to one lot of cooled sweet cream and to other lots 8 per cent was added at various periods before churning, the periods used in the eight trials including 3, 6, 16, 40, 64, 88 and 112 hours. Butter made with culture consistently scored higher than butter made without culture, both before and after storage. Holding periods greater than 16 hours were of no advantage. $\text{Amc} + \text{ac}_2$ contents of the cream at churning were sometimes increased and sometimes decreased by holding for the various periods with culture added.

Wiley *et al.* (137) found that addition of 0.5 per cent culture to pasteurized and chilled sweet cream, with holding overnight at a low temperature before churning, improved the flavor of butter. No acid developed in the cream under these conditions, although the resultant butter had a somewhat "brighter" flavor than if made from sweet cream alone.

Fabricius (31) stated that when culture is added to cream at 40° F. or below and the mixture held overnight at a low temperature, considerable amc usually is oxidized to ac_2 . Later (32), he noted that addition of culture to pasteurized and cooled cream several hours before churning brings about

² Although in the earlier review (43) temperatures are given on the Centigrade scale, the Fahrenheit scale is employed herein since so many of the results were reported on this basis.

³ Throughout the remainder of the review acetylmethylcarbinol = amc and diacetyl = ac_2 .

little change in the acidity of the cream but usually gives definite increases in the $\text{amc} + \text{ac}_2$ content.

Adding butter culture to pasteurized cream and ripening somewhat. Because of the danger of accelerating chemical deterioration in butter, extensive ripening of cream for salted butter is now rarely used. However, at temperatures favorable for the culture organisms, such as 70° F., slight ripening sometimes is employed, particularly with butter intended for immediate consumption. Ripening of the cream commonly is followed by cooling to a temperature which will largely prevent the action of the culture organisms, after which it is held for some hours before churning.

The Ontario Agricultural College (83) reported that an improved quality of butter for immediate consumption could be made from high acid cream by reducing the acidity, adding a good culture and ripening to a low acidity before churning.

Jackson (52) compared the scores of butter made by adding 10 per cent culture to neutralized and pasteurized cream and ripening for 30 minutes with the scores of butter made from neutralized and pasteurized cream without added culture; he found practically no difference.

White *et al.* (134) stated that ripening cream with culture, even to low acidities, improved the score of the fresh butter. In the experiments of Walts (131) butter made from neutralized and pasteurized cream with culture scored higher in all the trials than butter made without culture. The trials involved ripening the cream 2 hours at 65° F., ripening 12 hours at 65° F., ripening to 0.30 per cent acid at 65° F., ripening 1 hour at 48° to 56° F. and ripening overnight at 50° F.

According to Lucas *et al.* (65) the average score of fresh butter from cream ripened to 0.30 per cent acid was higher than that from cream to which culture was added at the time of churning, that from cream to which culture was added just before working, that from cream ripened to 0.45 per cent acid and that from sweet cream (without culture). Butter from sweet cream lacked flavor and that from cream ripened to 0.45 per cent acid had an undesirable acid flavor; after 30 days in storage, the latter had suggestions of fishy, unclean and tallowy flavors. The authors noted that even under ideal conditions it is unwise to ripen cream with culture, even moderately, if the butter is to be stored for any considerable period.

Price *et al.* (96) compared butter manufactured with and without culture. The culture was added to pasteurized and neutralized cream when it had been cooled to 70° F. After holding at this temperature until 0.28 to 0.30 per cent acid had developed, the cream was cooled to 35° to 40° F., held overnight and churned the following morning. Of 93 churnings, 59 were made with culture added to the cream and 34 were made without culture. The fresh butter made with culture ranged in score from 90.5 to 95

and averaged 93.03; butter made without culture ranged in score from 90.5 to 93.5 and averaged 91.96. After holding 1 month at 35° to 45° F., scores of butter made with culture ranged from 89 to 94 and averaged 92.07; scores of butter made without culture ranged from 89 to 94 and averaged 91.6. After holding 6 months at 0° to 10° F., scores of butter made with culture ranged from 90 to 94 and averaged 92.4; scores of butter made without culture ranged from 89.66 to 93.5 and averaged 91.63. From the frequency distribution of the butter scores, it was noted that 93.23 per cent of the scores of the fresh butter made with culture were 92 and above as compared to 61.77 per cent for fresh butter made without culture. After holding 1 month at 35° to 45° F., 61.02 per cent of the butter made with culture scored 92 and above as compared to 49.98 per cent for butter made without culture. After holding 6 months at 0° to 10° F., 77.97 per cent of the butter made with culture scored 92 and above as compared to 44.12 per cent for butter made without culture. There was very little difference between the keeping qualities of butter made with and without culture. The scores of butter made with culture decreased more during holding 1 month and 6 months, but at the end of each holding period a considerably larger percentage of scores were above 92 than in the case of butter made without culture.

Results of Overman (86, 87) showed that butter having the highest initial score was churned from fresh cream separated from fresh, sweet whole milk. Butter made from pasteurized sweet cream after ripening with culture and partially neutralizing scored higher when fresh than butter made from the same cream after spontaneous souring and neutralizing to the same acidity.

Fabricius and Hammer (33) compared addition of 8 and of 10 per cent culture to cream after cooling with addition of 8 per cent culture to the cream at 70° F., ripening for 1 hour and then cooling. Ripening of the cream gave butter more often high in score than addition of 8 or of 10 per cent culture to the cooled cream, when the butter was fresh, after 6 weeks at 28° F. and after 6 months at 0° F. Differences in score, however, were only significant in the case of the butter held 6 weeks at 28° F. Another series of comparisons involved: (a) Addition of 8 per cent culture to pasteurized and cooled cream with holding at 28° to 36° F.; (b) addition of 8 per cent culture to pasteurized and cooled cream with holding at 41° to 52° F.; and (c) addition of 8 per cent culture to pasteurized cream at 70° F. followed by ripening for 1 hour, cooling and holding at 28° to 36° F. Butter from cream to which 8 per cent culture was added with holding at 41° to 52° F. and from ripened cream was more often higher in score than butter from cream to which 8 per cent culture was added with holding at 28° to 36° F.; however, in no case was the difference significant.

Crossley (21) stated that when growth of the culture organisms is allowed to continue in cream after adding culture, production of ac_2 is in-

creased and full-flavored butter is obtained. The acid developed during ripening, however, has an adverse effect on the keeping qualities of the butter.

Langton (59) noted that cream must be ripened in order to obtain full-flavored butter. *Streptococcus lactis* or *Streptococcus cremoris* plus flavor organisms were considered essential. Various strains of lactic acid streptococci brought about appreciable differences in the flavor of butter.

According to Fabricius (31) when culture is added to cream at 70° F. and the mixture held 2 to 3 hours before cooling, the ac_2 content often decreases and the cream must then develop about 0.40 per cent acid before appreciable amounts of ac_2 are produced.

Adding butter culture to pasteurized cream at churning. Butter culture sometimes is added to pasteurized cream at the time of churning. From the standpoint of flavor of the butter, both experimental results and commercial experience indicate that this is less desirable than holding a mixture of cream and culture cold for some hours before churning, but it may be necessary with limited vat space. It also avoids risking an increase in the acidity of the cream-culture mixture when the cooling and holding equipment are inadequate.

Dean (28) noted that the flavor of butter made from sweet cream with culture added at churning was better than that of butter made from sweet cream alone. Sweet cream churned with 27.5 per cent added culture gave better butter than ripened cream when the butter was fresh; it also held its flavor better during storage. Rogers and Gray (104) found that butter made from pasteurized cream with culture added retained its fresh flavor better than ripened cream butter.

Mortensen (80) compared butter made from sweet cream and from sweet cream to which culture was added before churning. Immediately after the butter reached the market, that made from sweet cream scored higher in 8 of 32 trials, that made from sweet cream with culture added scored higher in 16 trials and the scores were the same in 8 trials. After 2 months in cold storage (0° F.), the sweet cream butter had the higher score in 10 of 35 trials, that made from sweet cream with culture added had the higher score in 20 trials and the scores were the same in 5 trials. After 9 months in cold storage, the sweet cream butter had the higher score in 11 of 22 trials, that made from sweet cream with culture added scored higher in 8 trials and the scores were the same in 3 trials. In another experiment, ripened cream butter was compared with butter made from sweet cream to which culture was added without ripening. Immediately after the butter reached the market, the ripened cream product had the higher score in 23 of 32 trials, that made from sweet cream with culture added scored higher in 6 trials, while the scores were the same in 3 trials. After 2 months in cold storage,

the ripened cream butter had the higher score in 10 of 35 trials, that made from sweet cream with culture added scored higher in 20 trials and the scores were the same in 5 trials. After 9 months in cold storage, the ripened cream butter had the higher score in 6 of 23 trials, that made from sweet cream with culture added scored higher in 16 trials and the scores were the same in 1 trial.

According to Grimes (37) butter made from sweet cream to which culture had been added without ripening had a higher initial score than butter made from sweet cream.

Lucas *et al.* (65) found that, after 30 days at 0° F., butter made from cream having culture added at churning scored highest, butter made with culture added just before working was second, butter made from cream ripened to 0.30 per cent acid was third, butter made from sweet cream (without culture) was fourth and butter made from cream ripened to 0.45 per cent acid was fifth. The butter made from cream ripened to 0.45 per cent acid declined in average score 1.7 points during the storage while that made from cream having culture added at churning decreased in average score 0.7 of a point. After 3 months in storage the average score of the butter made with addition of culture to the cream at churning was still highest; the butter was excellent and the average score had decreased 1.1 points. Of almost equal quality was the butter having culture added just before working, the average score having decreased only about 0.5 of a point. The sweet cream butter was third and the ripened cream butter was last.

Parsons (89) made butter from split vats of cream, with and without culture. The average scores of the fresh butter showed that culture improved the product. In the individual comparisons butter made with culture scored 0.20, 0.22, 0.27, 0.32 and 0.50 of a point higher than that made without culture. Butter made with culture appeared to be slightly more uniform and had a more pleasing aroma than butter made without culture. A wider difference in score in favor of butter made with culture was found with cream graded to make a 90 to 91 score product than with cream graded to make an 89 score product.

A comparison, by Fabricius and Hammer (33), of addition of culture to pasteurized and cooled, neutralized sour cream at churning and 16 hours before churning indicated that addition at churning consistently gave butter lower in score.

Crossley (21) found it desirable to make butter from sweet cream plus culture, rather than from ripened cream, in order to insure satisfactory keeping qualities during lengthy cold storage. When this procedure is followed, reliance is placed entirely on the flavor of the culture and well-ripened culture is of paramount importance.

Davies (23, 27) stated that the most promising method of using culture consists of employing small quantities of special cultures which are allowed

to produce large amounts of ac_2 ; the culture is added to the cooled cream and the mixture churned without delay.

According to Hunziker (48; p. 363) some buttermakers follow the practice of adding culture to the cream just before churning in order to avoid excessive acidities through cream ripening. Use of 5 to 7 per cent culture usually raises the cream acidity approximately 0.03 to 0.04 per cent. The flavor and aroma of the resulting butter depend solely on the degree to which they are present in the culture. Hunziker concluded that the effect of culture on the butter is slight, and it is questionable whether the practice is economically justifiable.

Jacobsen and Evans (53) obtained a slightly higher general level of scores on butter made with culture than on butter made without culture, when the butter was scored fresh.

Adding butter culture directly to butter. When cream with added culture is churned, a portion of the flavor constituents in the culture is lost in the buttermilk. Because of this undesirable partition, there have been various suggestions that culture be worked into the butter. With normal churning procedures the amount of culture that can be added in this way is limited, if the usual composition standards are to be met; with special churning procedures the amount can be increased. Various butter experts originally predicted that working culture into butter would have an undesirable effect on the keeping qualities; in general, this has not been the case but with an unsatisfactory culture there may be dangers along this line.

The Minnesota Agricultural Experiment Station (74) reported that working a small amount of culture into butter at the churn produced butter with a better flavor than that made from ripened cream. Lucas *et al.* (65) concluded that working culture into butter had practically no effect on the curd content or on deterioration. Bouska (10) suggested that the cost of culture can be reduced by working it into the butter.

Hunziker (48; p. 364) stated that working the desired aroma substance directly into butter eliminates much of the added operating expense, avoids the quality-jeopardizing effect of high churning acidities and prevents waste of the flavor principle. He suggested that instead of using water to wet the salt and bring the moisture content to the desired point, several gallons of culture be added to the butter with the salt. Culture so used assists somewhat in freshening the flavor of butter made from unripened cream. It does not increase the acidity of such butter sufficiently to augment the danger of deterioration due to chemical reactions. The keeping qualities of butter so treated are similar to those of butter made from unripened cream without addition of culture. The resulting improvement in flavor, however, is slight; the butter still lacks the full flavor and aroma of ripened cream butter.

Unsalted Butter

With unsalted butter intended for table use, a high flavor is very desirable and this necessitates high ripening of the cream, whether it is sweet or neutralized sour cream. Frequently the cream is ripened to 0.45 per cent acid or more, values which often would be disastrous with salted butter because of the greatly accelerating effect of the acid-salt combination on chemical changes in butter. In other cases much lower acidities are employed. Some plants use lower ripening acidities in summer than in winter because during the warmer weather there is more opportunity for development of the culture organisms in the butter itself and this may lead to their overdevelopment, with resulting destruction of flavor constituents (43; p. 115).

High ripening of cream for unsalted butter commonly is carried out at relatively low temperatures, with the temperature often changing during the ripening period as the cream warms or cools from the air of the room. Frequently the temperature of the cream is adjusted to a point which will give the desired acidity during the normal holding period, with the usual inoculation and usual room temperature; in general, the point to which it is reduced is lower in summer than in winter and there may be a shift from day to day as the acidities developed are too high or too low.

Experiments conducted by Jackson (52) indicated that when cream was inoculated with 10 per cent culture, after being neutralized and pasteurized, and ripened 30 minutes at 70° F., the resulting unsalted butter was lower in score than that made from neutralized and pasteurized cream without added culture.

Hunziker (48; p. 363) stated that, with unsalted butter, it is desirable to ripen the cream to a full flavor and aroma. Excellent results are obtained by low-temperature ripening, such as 50° to 55° F., overnight. With this method the cream usually shows a pronounced flavor and aroma the following morning and an acidity of about 0.35 to 0.45 per cent. With ripening at higher temperatures (60° to 70° F.), the aroma character tends to be somewhat more pronounced and the acidity somewhat higher (0.5 to 0.65%).

Hedrick and Hammer (45) studied the effect of ripening cream to various acidities on its ac_2 and amc contents. Results of ten semi-commercial trials on cream (seven on sweet cream, three on neutralized sour cream) ripened to various acidities at temperatures from 50° to 55° F. showed an increase in ac_2 with an increase in acidity in nine trials (90%) and in amc in seven trials (70%). Some of the increases were small. Ac_2 decreased in one trial (10%), the drop being from 1.88 to 1.49 ppm. with a change in acidity from 0.37 to 0.49 per cent, and amc in three trials (30%).

With unsalted butter that is being stored for reconstitution into cream, ice cream mix, etc., small amounts of culture sometimes have been used, the idea being to improve the keeping qualities of the product. The culture has

been added at churning, or some hours before churning when the cream temperature is low, a significant increase in acid being carefully avoided.

USE OF SPECIAL CULTURES IN BUTTER MANUFACTURE

Special cultures, instead of the usual butter cultures, occasionally have been employed in butter manufacture, mainly on an experimental basis. They include pure cultures of the normal butter culture organisms and of various closely related species, in some instances the pure cultures being given special treatments intended to intensify flavor development in them, and also butter cultures grown under special conditions. Certain of the special cultures eventually may prove of value in commercial butter manufacture.

Hammer (38) found that addition of *Streptococcus citrovorus* and sterile lactic or citric acid to pasteurized cream resulted in a significant increase in score of the resulting butter; the increase over the control was greater with 0.2 per cent citric acid than with 0.1 per cent. Without added acid the organism gave a slight increase in score. Similar results were obtained with *Streptococcus paracitrovorus*. *S. lactis* gave a better flavor and aroma with sterile citric acid added to the cream than without the acid, but the flavor and aroma of the resulting butter were not as good as those produced by *S. citrovorus* and added citric acid. Addition of sterile citric acid without any inoculation gave a higher score than when *S. lactis* was used without added acid. Later, Hammer (39) noted that considerable flavor and aroma could be produced in butter by growing one of the flavor organisms (*S. citrovorus* or *S. paracitrovorus*) alone in cream that had been carefully pasteurized. A small amount of citric acid added to the cream before inoculation resulted in excellent flavor in the butter.

Bogdanow (8) made butter with *S. lactis*, with *S. cremoris* and with flavor organisms. The best flavor was obtained with *S. lactis*; however, the butter showed the least storage capacity. Butter made with flavor organisms alone had no flavor when fresh and scored lowest.

In the experiments of Maddock (66), butter of exceptionally fine flavor was produced in laboratory tests by use of pure cultures of *S. paracitrovorus*. However, under practical conditions the uncertainties were too great to warrant its general use, the organism being unable to compete successfully with the contaminants encountered in practice.

Fabricius and Hammer (33) compared the use of no culture, regular butter culture and modified butter culture which was prepared as follows: Whole milk was heated to 180° F. for 1 hour, cooled to 70° F. and inoculated with a pure culture of *S. paracitrovorus* in sterile milk. After incubating 24 hours at 70° F., 0.30 per cent sulfuric acid and 0.15 per cent citric acid were added. The culture was incubated another 24 hours at 70° F. and then cooled to 40° F. With sweet cream, modified culture gave butter sig-

nificantly higher in score than regular culture when the butter was fresh and also after holding a few weeks at 28° F. Regular culture gave butter significantly higher in score than no culture when the butter was fresh; after holding at 28° F. the difference was not significant, although the higher score was more often obtained with the culture. Modified culture gave butter significantly higher in score than no culture when the butter was fresh and also after holding at 28° F. Trials involving sour cream showed identical results. Comparisons also were made using no culture, regular culture and regular culture neutralized to 0.30 per cent acid. With sweet cream, regular culture and regular culture neutralized gave butter that did not differ significantly in score when fresh or after cold storage; after holding at 28° F. the butter made with neutralized culture was significantly higher in score. Butter made with regular culture was significantly higher in score than that made without culture when fresh, whereas after holding at 28° F. or after cold storage the difference was not significant. Butter made with regular culture neutralized was significantly higher in score than that made with no culture when fresh and after holding at 28° F., but after cold storage the difference was not significant. Trials involving comparisons of no culture, modified culture and modified culture neutralized were made with sweet cream. Butter made with modified culture was more often higher in score than that made with modified culture neutralized, both after holding at 28° F. and after cold storage; when scored fresh the numbers of higher scores were equal. Differences were significant only with the cold storage butter. Modified culture and also modified culture neutralized gave butter significantly higher in score than no culture in all cases. However, the difference was significant only in case of the fresh butter. Another series of trials involved adding regular butter culture and pasteurized butter culture to sweet cream. The pasteurized culture was made by heating ripened culture to 145° F. for 30 minutes and filtering off the curd. The serum was cooled to 40° F. and used in cream. Butter made with regular culture was more often higher in score than butter made with pasteurized culture when fresh, after holding at 28° F. and after cold storage; differences were significant when the butter was fresh and after holding at 28° F.

Matuszewski *et al.* (69) made laboratory churnings using 5 per cent of a culture of *Streptococcus diacetylactis* to ripen the cream to about 0.5 per cent acid at 68° F. Flavor of the butter was good, but at times was too high, especially after 3 to 5 days at 50° to 59° F. When *S. diacetylactis* was used in combination with *S. cremoris* and *S. citrovorus*, there was an improvement in the butter compared to butter made with regular culture.

Comparisons were made by Brewer *et al.* (11, 12) to determine the quality of butter produced by use of aerated-pressure butter culture. These usually were prepared by adding 2 per cent active culture to pasteurized skim milk, although in some cases whole milk was used to prevent excessive

foaming during aeration. After inoculation the milk was divided, one portion being ripened with aeration under 60 pounds pressure, while the other was ripened as usual to serve as a control. The ripened cultures were cooled and then added to divided lots of pasteurized sweet cream. After appropriate holding, the mixtures were churned. Results showed that aerated-pressure culture gave butter scoring 0.15 to 1 point higher than that made with regular culture; with the aerated-pressure culture the flavor was higher with both sweet and sour cream butter. In trials in which 5 per cent regular culture and only 2 per cent aerated-pressure culture were added to cream, the butter made with the aerated-pressure culture regularly had the higher flavor.

Khubebandani (55) found that use of *S. citrovorus* cultures, treated with citric acid or sodium citrate 24 hours prior to inoculation into cream, gave better flavor and aroma in butter than use of untreated cultures. The keeping qualities of such butter were not affected by the citric acid or sodium citrate.

Davies (27) attempted to produce butter of average flavor and low acidity by use of pure cultures of *Betacoccus cremoris* and *S. paracitrovorus*. The cultures were employed successfully in the laboratory but became contaminated in plants and finally were essentially regular butter cultures.

SURVIVAL AND GROWTH OF BUTTER CULTURE ORGANISMS IN BUTTER

Rapid growth of the butter culture organisms in milk, cream, etc., at favorable temperatures suggests that they would grow rapidly in butter. However, in the case of salted butter the added salt is an important inhibiting factor and with both salted and unsalted butter the low holding temperatures often used and the small amounts of milk solids-not-fat present also are of significance in limiting the growth. Butter culture organisms sometimes survive for extended periods in butter.

Salted Butter

Sayer *et al.* (109) found a rather regular decrease in the numbers of lactic acid bacteria in butter during storage at 41° F. The decrease was related to the concentration of salt in the butter, higher concentrations giving greater decreases. Apparently the salt content was not the only reason for the decrease in numbers.

Washburn and Dahlberg (133) reported that *S. lactis* withstood salt and low storage temperatures better than any other organism in butter. It was the only species appearing regularly in all lots of butter. After 304 days in storage, every lot of salted butter contained essentially only *S. lactis*. Brown *et al.* (15) noted that lactic acid bacteria persisted in butter for as long as 275 days (in one case 426 days). According to the authors salt and low temperatures may cause such a slow rate of metabolism that relatively

little acid is produced and life of the cell is prolonged. Butter in storage showed a gradual decrease in the amount of lactose and a gradual increase in acidity.

Grimes (37) found that butter made from cream to which culture had been added, either without ripening or with ripening to various acidities, retained per ml. from 0.5 to 2.0 per cent of the culture organisms per ml. of the cream-culture mixture. The flora of the butter consisted of *S. lactis* and *S. paracitrovorus*, *S. lactis* making up 70 to 100 per cent of it. After 6 months at -6° F. over 98 per cent of the bacteria had died. After cold storage, sweet cream butter often had a higher bacterial count than butter made from cream to which culture had been added. In general, it appeared that the normal flora of pasteurized cream found an acid environment unfavorable; also, utilization of lactose by *S. lactis* and *S. paracitrovorus* was thought to be a factor. The flora of the butter when taken out of cold storage consisted chiefly of *S. lactis* and acid-forming, non-coagulating species. *S. paracitrovorus* usually was present. *S. lactis* was not found in pasteurized cream butter made without culture when first manufactured but was found after cold storage.

It was noted by Parfitt (88) that salted butter made with culture showed less variation in pH during storage than butter made without culture.

In the trials of Nelson and Hammer (81) butter was churned under both laboratory and commercial conditions. With the laboratory butter, one series of trials involved butter made from cream of poor quality so that the microscopic count of the fresh control butter was high, considerable numbers of both streptococci and other organisms being present. During holding at 69.8° F. the numbers of organisms in the control butter showed comparatively little change. With three of the organisms (butter culture types) used in the cream, the butter showed fluctuations in the numbers of streptococci during the holding in both the plate and microscopic counts, but there were no definite trends evident. With the fourth culture organism used, each method of counting indicated a definite increase in the numbers of streptococci. Throughout the examinations the microscopic counts regularly were much higher than the plate counts with both the streptococci and the other organisms, and the ratio between the two was extremely variable. Another series of trials involved cream of very good quality and the microscopic and plate counts on the fresh control butter were low and did not show significant changes during the holding. With the butter made from cream inoculated with culture organisms, there was a general tendency for a decrease in the numbers of streptococci with both methods of counting, and the decrease definitely was greater on a percentage basis with the plate method than with the microscopic method. With butter made under commercial conditions, the general change in the numbers of streptococci, as shown by the microscopic counts, was a decrease that commonly was exten-

sive; with certain samples it was very regular while with others some fluctuation occurred. With several samples there was a suggestion of a small increase in the numbers of streptococci after 1 day, but the evidence was not at all convincing. Plate counts showed the same general changes in numbers of organisms. The streptococci appeared to be greatly autolyzed and the chains seemed to be shorter after 7 days than after 2 days.

Unsalted Butter

Rahn *et al.* (100) noted that all lots of butter made without salt and stored at 42.8° F. showed a very high increase in acidity, compared to lots of salted butter. When held at 21.2° F. the unsalted butter showed a slightly higher increase in acidity than the salted butter.

Nelson and Hammer (81) found a definite development of the butter culture streptococci in unsalted butter held at a favorable growth temperature. It was evident from both plate and microscopic counts on butter prepared in the laboratory, using a pure culture of *S. lactis* or one of the flavor organisms, and also on butter prepared under commercial conditions with butter culture. Growth resulted in the presence of long chains of streptococci and these were striking evidence of multiplication since they were not found in the salted butter or in butter (salted or unsalted) made without addition of organisms to the pasteurized cream. In the unsalted butter there also was extensive growth of organisms other than streptococci, as shown by both plate and microscopic counts.

Hammer (42) noted that in unsalted butter made with butter culture and held at 69.8° F., acidity of the serum increased more rapidly and reached higher final values in underworked butter than in thoroughly worked butter; the relationship was evident with either a small or a large inoculation of the cream.

Effect of reworking on changes in the pH of serum obtained from unsalted butter made with culture was investigated by Long and Hammer (64); pH determinations were made at once, at reworking and at different periods following the reworking. In the laboratory studies, some trials involved underworked and moderately worked butter, while others involved moderately worked and thoroughly worked butter. The more the control butter was worked, the slower were the decreases in pH of the serum. Reworking regularly resulted in a more rapid decrease in pH of the serum, and this was evident at each examination. In two trials on underworked and moderately worked butter the differences were considerable, while in one trial, also on underworked and moderately worked butter, the differences were less extensive. In one trial the control portion of the thoroughly worked butter showed relatively little decrease in pH of the serum throughout the holding period, while the reworked portion showed considerable change; the final pH value of the serum of the reworked portion was rela-

tively high compared to those of underworked and moderately worked butter similarly treated, suggesting that thorough working of the original butter may tend to counteract the effect of reworking. Butter churned under commercial conditions with a relatively large amount of culture in the cream showed the same general decrease in pH of the serum as butter made under laboratory conditions.

SOURCES OF NATURAL BUTTER FLAVOR

With butter made from raw cream allowed to sour spontaneously, the flavor is largely determined by the changes that go on during the holding of the cream, particularly those due to micro-organisms. When the fermentation is favorable the flavor of the butter is desirable, and when it is objectionable the flavor of the butter is undesirable. A desirable flavor is due to action of certain types of bacteria which include the species normally present in butter cultures and perhaps others as well. Actually, butter cultures were developed with the idea of duplicating the flavor of the best of the butter made from naturally soured cream.

Butter made without culture from cream in which there has been no appreciable bacterial action has a flavor which is due to the milk constituents, particularly the fat, plus flavoring materials absorbed or added during production and handling of the milk, cream or butter. The feed, season, stage of lactation of the cows and various other factors may influence the composition of the milk and the flavor of butter made from it. Even slight bacterial action in the milk or cream may modify the flavor. For many consumers, proper use of butter culture definitely improves the flavor.

Conn (18) noted that the flavor of butter is not due to butterfat but to certain volatile products present in small quantities. The odors produced by various bacteria plainly indicated that volatile substances were being produced during the ripening of the cream as a result of the fermentation. It was impossible to notice the odors produced by the growth of various species of bacteria without being convinced that they had something to do with the superior flavor of butter made from ripened cream.

Ruehle (108) indicated that the flavor of butter is dependent on the type of fermentation which occurs in the cream previous to churning. Hammer (41) stated that the desirable flavor and aroma of butter come from two main sources. With butter made from cream in which there has been no development of micro-organisms, the desirable flavor comes from the milk constituents present, particularly the fat, while with butter in whose manufacture culture has been used, the desirable flavor comes from the milk constituents and also from the products formed by the culture organisms. Ruehe (106) suggested that the flavor and aroma of butter are the grand total of the natural flavor of fat as it is normally produced by the cow, plus the flavors of the ingredients added during manufacture, plus the flavors

and odors absorbed during handling and storage, plus flavors caused by activity of micro-organisms, plus flavors which result from chemical changes in the product. Neuberger (82) noted that the amount of butter aroma depends on the season of the year and feed of the cows.

Another general comment (103) stated that the flavor of butter is due primarily to the natural flavor of the cream from which it is churned. Butter made from cream in which there has been no bacterial fermentation has a characteristic flavor, agreeable but mild. The nature of the flavor is dependent to some extent on the feed and may be affected unfavorably by ingestion of highly-flavored feeds of various kinds. Fat is a solvent for many flavor and aroma-producing substances and the odors and flavors to which milk or cream may be exposed, as well as those formed in the serum by bacteria, yeasts or molds, may be carried into the butter.

Results obtained by Sherwood and Hammer (113) suggested that seasonal variations in flavor and aroma of butter are not related to differences in the citric acid content of the milk or cream. Experiments conducted by Steuart (118) indicated that the composition of the fat is an important factor in the production of aroma.

EFFECT OF BUTTER CULTURE ON FLAVOR OF BUTTER

Following the introduction of butter cultures, their use spread rapidly, which definitely indicates they are of value in improving the flavor of butter. Many plants continue to use culture even when there are serious difficulties in the procurement of milk for it. In others, use of culture has been discontinued, the idea often being that culture increases the cost of butter manufacture and that the flavor improvement obtained is not reflected in a price increase.

Over the years there have been various reports covering the effect of culture on the flavor of butter. A number of these are reviewed in the consideration of the methods of using butter cultures in the manufacture of butter but there are others of significance.

Bouska (10) indicated that if butter made without culture lacks aroma, it is handicapped in maintaining its advantage as a spread for bread in competition with substitutes.

Wilster (138) stated that many experienced creamerymen and butter dealers believe the use of fine-flavored culture in first-grade, neutralized sour cream and in sweet cream results in butter which scores higher in flavor than that made without culture. When butter is sent to a scoring contest, culture is used with the majority of the churnings. The average score of butter made with culture usually is higher than that of butter made without culture. In a cold storage contest of the National Creamery Buttermakers' Association at St. Paul, Minnesota, in 1924, culture butter averaged 0.93 of a point higher in score when fresh than butter made without culture. In a

similar contest at Cleveland, Ohio, in 1928, culture butter averaged 0.65 of a point higher in score when fresh than butter made without culture. At the Pacific International Dairy Products Show at Portland, Oregon, in 1931, butter made with culture averaged 1.40 points higher in score than butter made without culture. At the Oregon Butter and Ice Cream Makers' Annual Convention at Corvallis, Oregon, in 1932, butter made with culture averaged 0.67 of a point higher in score than butter made without culture. At the Pacific International Dairy Products Show at Portland, Oregon, in 1935, butter made with culture averaged 0.80 of a point higher in score than butter made without culture.

Fabricius (31) listed the arguments in favor of use of butter culture as follows: (a) Overcomes heated flavor, (b) decreases lime and burnt neutralizer flavor, (c) helps to overcome some feed defects, (d) may help to overcome tallowy flavors and (e) in unsalted butter its use decreases danger of cheesy, putrid and surface taint flavors. The arguments against the use of butter culture were listed as follows: (a) Cost, (b) improper use may cause oily and fishy flavors, (c) accentuates some feed flavors, (d) good culture is difficult to make and (e) price differential on the market does not justify its use.

Hoecker and Hammer (47) found that butter made with butter culture, *S. citrovorus*, *S. paracitrovorus* or *Streptococcus citrophilus*, usually contained relatively large amounts of ac_2 and amc and commonly placed high in a series of churnings. However, butter containing exceptionally large amounts of ac_2 and amc sometimes placed low and was criticized as being coarse, sour, oily or containing some other objectionable flavor. Butter made with *S. diacetilactis* usually contained large amounts of ac_2 and amc but often was placed low because of off flavors present. Butter made without culture or with *Streptococcus aromaticus* contained only small amounts of ac_2 and amc and usually placed low.

EFFECT OF BUTTER CULTURE ON KEEPING QUALITIES OF BUTTER

Consideration of the effect of butter culture on the keeping qualities of butter involves two distinct relationships. The first is the effect on the general score and concerns largely chemical changes in the butter, although with some lots of butter bacterial changes also are of significance, while the second is the effect in preventing certain bacterial defects of butter.

On General Score

Use of culture in butter manufacture gives the product more flavor but, because of the influence of serum acidity on chemical changes, it may result in serious deterioration. In salted butter a high flavor due to culture and good keeping qualities over long periods are more or less incompatible. Present methods of using culture are intended to give as much flavor im-

provement as possible without seriously affecting keeping qualities, the period the butter is to be held being considered.

It should be recognized that serum acidity is only one of the factors involved in chemical deterioration in butter and that some of the changes attributed to acid may have been greatly influenced by some other factor, such as contamination with copper. A serum acidity which does not result in significant deterioration when the copper content of the butter is low may be very serious when the copper content is high (50).

The early studies of Rogers and Gray (104) indicated that butter made from pasteurized cream with culture added does not have as good keeping qualities as that made without culture. A report from the Ontario Agricultural College (83) suggested that butter of improved quality may be made from high acid cream by neutralization and addition of good culture. A low churning acidity was more favorable to good keeping qualities in butter than a high churning acidity.

Mortensen (80) found that butter made from sweet cream with culture added scored higher after 2 months at 0° F. than butter made without culture, while after 9 months it scored lower. Butter made from sweet cream with culture added scored higher after 2 months and also after 9 months than butter made from ripened cream.

White *et al.* (134) stated that the improvement in flavor of fresh butter resulting from addition of culture to the cream usually is lost when the butter is placed in cold storage.

According to Lucas *et al.* (65) addition of a moderate amount of culture to cream before churning, or to the butter at the time of working, caused the butter to score higher after 3 months at 0° F. than if none had been used. Whether the aroma due to the culture covered the slight off flavors that normally develop in sweet cream butter during storage, or whether some inhibitory action took place when culture was used, the authors were unable to say. Ripened cream butter deteriorated more rapidly during storage than butter from sweet cream, butter from sweet cream with culture added just before churning or butter to which culture was added at the time of working. No relationship was found between use of culture and the iodine number of butterfat from the butter. The increase in acidity of cream obtained by using culture seemed to cause a slight increase in the acid number of the fat.

Results obtained by Hammer and Jensen (44) indicated no evident relationship between acidity of butter culture and quality and keeping qualities of butter made with it from sweet cream pasteurized at 145° F. for 30 minutes. When fresh, and also after storage, butter made with high acid culture and butter made with low acid culture ranked high or low approximately the same number of times. The small variations noted in the deterioration of the butter in storage seemed to be due to much or little

deterioration in one or two lots and did not regularly follow differences in acidities of the cultures. There was no evident relationship between acidity of the culture and quality and keeping qualities of butter made from neutralized sour cream pasteurized at 145° F. for 30 minutes. A somewhat greater variation was noted in the deterioration during storage than with butter made from sweet cream, and the difference was most conspicuous in the butter made with high acid culture; however, the variation did not follow the acidities of the cultures. Acidity of the culture would not be expected to have a great effect on the quality of fresh butter made by adding 10 per cent culture to pasteurized cream without ripening. With this method of manufacture, the flavor and aroma of the butter are directly influenced by the flavor and aroma of the culture, so that with cultures of good quality variations in acidities would be comparatively unimportant. Neither would large variations in the deterioration of the butter be expected to follow variations in acidities of the cultures because the great dilution of the added culture with cream of a much lower acidity results in only very small differences in the acidities of the butter serum. Butter made from neutralized sour cream showed a somewhat greater variation in score decreases during storage than butter made from sweet cream; this may have been due to differences in the quality of the cream.

Parsons (89) found little difference in keeping qualities of butter made with and without culture.

Results of Overman (86, 87) showed that the lots of butter which held their score best were churned from fresh cream separated from fresh, sweet milk; they were not salted and were salable after about 2 years in storage. Butter made from pasteurized sweet cream after ripening with culture and then partially neutralizing was correspondingly better than butter from the same lots of cream after spontaneous souring and neutralizing to the same acidity. Butter made from cream ripened with culture to 0.44 per cent acid and churned without neutralization scored higher and held its score better in storage than butter from the same lots of cream after ripening with culture to 0.61 per cent acid and churning without neutralization; the latter was correspondingly better than butter from the same lots of cream after ripening with culture to 0.82 per cent acid and churning without neutralization.

Bogdanow (8) reported that ripening cream with culture decreases the storage capacity of the butter.

Templeton (121) found that the storage qualities of butter made from cream in which culture, containing added citric acid or sodium citrate, had been used were very good. This was indicated by the fact that numerous samples which had been in storage for 6 weeks received higher scores than when fresh.

Wilster (138) noted that in the scoring contest conducted by the National Creamery Buttermakers' Association at St. Paul, Minnesota, in 1924, butter

made with culture scored 0.87 of a point higher after 4 months in storage than butter made without culture. In a similar contest at Cleveland, Ohio, in 1928, butter made with culture scored 0.61 of a point higher after 4 months in storage than butter made without culture. Wilster stated that approximately 100,000 pounds of butter are made annually in the Oregon State College creamery. Culture always is added to the cream and the cream is ripened to a serum acidity of 0.42 to 0.44 per cent. About 15,000 to 20,000 pounds of butter are stored during the summer for consumption in the fall. No objectionable flavors had developed in the butter made during the 6 years in which culture had been employed. There was no marked decrease in the score of butter made with or without culture during 1 month at 35° to 45° F. However, a decrease in score was observed when butter was stored 6 months at 0° to 10° F. The average decrease in score of the butter made without culture was 0.57 of a point. The average decreases for butter made with culture were 1.15 points when the cream was ripened, 0.74 of a point when culture was added and the cream held cold 16 hours previous to churning and 0.95 of a point when culture was added and the cream churned immediately; the average scores after 6 months, compared to that of butter made without culture, were 0.30, 0.66 and -0.06 of a point in favor of the respective methods of using culture. In one series of churnings, butter was made from 93 lots of cream, culture being used with 59 and no culture with 34. When the butter was stored 1 month at 35° to 45° F. and 6 months at 0° to 10° F., the average scores of the butter made with culture were higher than those of the butter made without culture. Chief comments of the judges on the butter made with culture were "creamy flavor," "sweet and clean," "fine aroma" and "fine flavor"; those on the butter made without culture were "flat," "lacking character," "insipid" and "tallowy." (See also 96.)

The results of Wiley (135) indicated that the presence of culture organisms in butter favored oxidation of the fat at storage temperatures. The oxidation that occurred in the presence of culture organisms, even when the acid produced in the culture was neutralized before churning, demonstrated the presence of some fat-oxidizing system in the ripened cream. This was believed due to some product of bacterial metabolism acting as a pro-oxidant; if this is the case, the product is not amc or ac_2 . Wiley considered the results may be explained by assuming that the lactic acid streptococci of the culture contain a fat-oxidizing enzyme which is most active at low pH and high salt concentration. Several mixed cultures (*S. cremoris* and betacocci) and a single strain of *S. cremoris* all increased oxidation, as compared to simple acidification of the cream.

According to Hunziker (48; p. 357) there are certain conditions of butter manufacture which permit controlled ripening without jeopardizing keeping qualities, and there are still other conditions of manufacture and distribu-

tion under which ripening to a full aroma and flavor is definitely beneficial to keeping qualities. The extent to which cream may be ripened and still have the butter reach the consumer without objectionable flavor deterioration due to chemical changes was considered to be influenced by such factors as: (a) Condition and quality of the original cream, (b) temperature at which butter is held between churn and consumer, (c) whether butter is intended for fresh consumption or for cold storage, (d) whether the cream is used for salted or unsalted butter and (e) salt concentration in salted butter. Hunziker (48; p. 353) also stated that cream ripening does not improve the chemical stability of butter and that, under average commercial conditions of manufacture, the ripening of cream to a full flavor and aroma shortens the life of salted butter. Usual flavor defects which develop with age in salted butter made from fully ripened cream were said to be oily-metallic, fishy and sometimes tallowy flavors. Salted butter made from sweet, unripened cream or from sour cream neutralized and pasteurized was said to keep better, from the standpoint of flavor deterioration due to chemical causes, than salted butter made from the same cream ripened to full flavor and aroma.

Jacobsen and Evans (53) found that butter made from first-grade cream without culture had better keeping qualities than butter made from first-grade cream with culture; the butter was scored after 3 and 5 months at 32° to 36° F.

In Preventing Certain Bacterial Defects

Growth of various bacteria responsible for development of specific defects in butter apparently often is delayed or prevented by use of butter culture in the cream. The inhibitory effect apparently is due not only to the products formed by the culture organisms but also to the presence of the organisms themselves.

Orla-Jensen (84) inoculated butter with *Pseudomonas fluorescens* and *S. lactis*. He noted that the lactic acid formed by *S. lactis* inhibited fat hydrolysis; there was little free volatile acid produced and the odor and taste of the butter were not especially objectionable.

Rogers (102) stated that during ripening of cream the lactose is partially fermented to lactic and similar acids which protect the butter from fermentation by less acid-tolerant organisms. The putrefactive bacteria, which often attack the curd of butter, usually were checked by the acid produced during cream ripening.

Results obtained by Mazé (70) indicated that micro-organisms causing deterioration in butter largely attack the casein and lactose. They were found to be retarded by lactic acid. Addition of lactic acid to butter at the rate of 0.5 to 1.0 g. per l. was suggested as a control measure. Lactic acid was not believed to completely prevent growth.

Shutt (114) reported that churning cream at an acidity of not less than 0.35 per cent was advantageous in preventing defects caused by *Ps. fluorescens* since it grows only feebly at a pH of 6.6. He was of the opinion that surface taint could be caused by *Ps. fluorescens*, but that it occurred only in sweet cream or neutralized cream butter and never in sour cream butter.

Virtanen (127) noted that water types of micro-organisms, which frequently cause cheesy and putrid defects in butter, are inhibited by the acid of sour cream butter.

Derby and Hammer (30) made butter from pasteurized cream inoculated with surface taint butter and studied the influence of salt and butter culture on production of the defect. Unsalted and low salted butter made without the culture developed surface taint in 2 days at 70° F., but medium salted butter made without the culture, unsalted butter made with the culture and salted butter made with the culture was normal after 7 days. Claydon and Hammer (17) added *Pseudomonas putrefaciens* to pasteurized cream and churned the cream with various amounts of butter culture added. None of the unsalted or salted butter made with the culture developed the putrid defect in 6 days at 70° F. but that made without the culture became markedly putrid. Five per cent culture inhibited the defect as effectively as 12 per cent. Results indicated that butter culture had an inhibiting effect on development of the putrid defect in unsalted butter when *Ps. putrefaciens* gained entrance to the butter either from cream or from wash water.

According to Knudsen (57) ripening cream with butter culture protects the resulting butter from attack by putrefactive bacteria which may get into it from water, but which cannot develop because the reaction is acid; ripening the cream with culture promotes development of certain defects, particularly in storage. Minster (75) considered the presence of a few lactic acid bacteria in unsalted butter as beneficial, since they tended to keep down growth of objectionable species.

In the trials of Hussong *et al.* (49) butter made from pasteurized cream inoculated with *Pseudomonas fragi* developed a rancid flavor somewhat less rapidly when 10 per cent butter culture was added to the cream just before churning than when the butter was made without culture.

St. von Nyiredy (119) reported that in butter made from ripened cream, coliform, proteolytic and *Ps. fluorescens* organisms showed practically no growth and decreased rapidly, while they increased markedly in sweet cream butter. The ripened cream butter had a pH of 4.6 to 4.8 and the sweet cream butter a pH of 6.4 to 6.8. It was concluded that failure of these groups to grow in ripened cream butter was due to the low pH.

Hunziker (48; p. 295) stated that efficient pasteurization of the cream, preferably followed by proper ripening with good culture, not only retards the general age deterioration which is characteristic of all unsalted butter but prevents the early appearance of the more serious flavor defects result-

ing from the presence in cream of specific flavor-damaging organisms. When unsalted butter was made from cream ripened to full flavor and aroma, bacterial deterioration was very noticeably retarded by the predominance of lactic acid bacteria and the relatively high acidity. These agencies assisted in checking the organisms which, if present, caused bacterial flavor defects in unsalted butter (48; p. 360). In comments on cheesy flavor, Hunziker (48; p. 668) noted that resistance of unsalted butter to bacterial age deterioration and to development of cheesy flavor is materially improved by ripening the cream with a good culture to a pronounced culture character and a reasonably high churning acidity (preferably about 0.35 to 0.45 per cent). It appeared that the great predominance of culture bacteria and the products of cream ripening are more or less antagonistic to the organisms responsible for cheesy flavor and definitely assist in holding them in check.

Fouts (35) studied the effect of butter culture and lactic acid on hydrolysis of fat in cream by pure cultures of lipolytic bacteria. Butter culture added to the cream definitely inhibited *Achromobacter lipolyticum*, *Alcaligenes lipolyticus* and *Ps. fluorescens*. When lactic acid was added to the cream used, all the organisms grew, even with enough acid to give a titrable acidity of 1 per cent.

Results obtained by Itzerott (51) showed that cream acidities below 0.15 per cent had little effect on the period required for development of the putrid defect in butter. Above 0.15 per cent, however, the acidity appeared to have a retarding influence. High acidities and low temperatures definitely inhibited the defect. Unsalted butter made from cream with 0.20 per cent acid and inoculated with rabbit organisms showed no evidence of the defect when held 3 weeks at 40° to 55° F. At higher temperatures (80° to 90° F.) the taint developed, even with a high acidity.

Pont (95) indicated that, within the range of safe limits from the standpoint of chemical changes in butter, high acidities aided in minimizing the putrid defect of butter.

Effect of butter culture on the ability of 52 fluorescent bacteria to produce defects in experimental butter was studied by Garrison and Hammer (36). Addition of 10 per cent culture to the cream prevented development of off flavors in unsalted butter by 16 of the cultures and in salted butter by 27 of them.

RELATIONSHIP OF SPECIFIC CHEMICAL COMPOUNDS TO DESIRABLE FLAVOR OF BUTTER

The desirable flavor produced in butter through use of butter culture is dependent on various compounds, some of which still may not have been identified. Ac_2 apparently is the most important of these but others, especially volatile acids, also are of significance. Ac_2 and volatile acid are

produced in butter culture, and also in ripening cream, primarily from citric acid by the normal flavor organisms while lactic acid is produced primarily from lactose by *S. lactis* (43; p. 84).

Volatile Acids

In 1890, Conn (19) stated that it usually is believed the flavor of butter is due to certain volatile fatty acids formed during the ripening of cream. The acids considered to be important were butyric, caproic, caprylic and capric.

Ferris *et al.* (34) determined the volatile acids in 14 lots of commercial sweet cream butter scoring 93 to 94. The values, expressed as ml. N/10 acid per 100 g., ranged from 0.2 to 0.4 by steam distillation and from 0.5 to 0.8 by direct distillation. The average score of the lots after storage 5 to 6 months at 22° F. was about 1 point lower than when they were fresh and the volatile acid values were about twice as large. After 6 to 7 months, the butter was removed from storage and kept 2 weeks at about 59° F. This caused a decided drop in score but no significant increase in volatile acid. More volatile acid was found in butter made from clean acid cream than in butter made from sweet cream.

Lind (63) determined the volatile acidity of butter by adding 150 ml. water and 15 ml. N/1 sulfuric acid to 100 g. butter and distilling with steam. From values obtained on skimmed milk, pasteurized cream and butter, the author concluded that butter dissolves a part of the volatile acid formed by the bacteria of the culture employed for acidification of the cream.

Results of Steuart (118) indicated that when full-flavored butter is distilled with steam, free butyric acid can be identified in the distillate as well as a neutral compound yielding butyric acid on saponification. Use in margarine of cultures producing butyric acid or addition to margarine of ethyl butyrate or butyric acid gave unsatisfactory results. Butyric acid itself was too fugitive, and a search was made for a parent substance which would continuously yield traces of the acid. In experiments with margarine prepared with skimmilk properly soured by lactic acid and aroma bacteria, it was found that a small addition of tri-butylin caused the mass to be gradually pervaded by a pleasant butter aroma. Unfortunately, the flavor was masked by bitterness of the tri-butylin. This compound was obtained from various sources and was synthesized, fractionated and refined, but in no instance was the bitterness avoided. Glycol di-butylin produced a similar aroma in margarine but also had an inherent bad flavor. Mixed butyric tri-glycerides were then prepared and the flavor of these indicated that the butyric acid in butterfat must be present as a mono-butyric tri-glyceride, with butyric acid in the beta position. The bitterness of tri-butylin is due to butyric acid in the alpha position. Coconut oil was butyrised by heating with butyric acid at 320° F. After treatment for 2

hours the neutralized fat gave Gilmour butyric numbers equivalent to 3 per cent butter and after treatment for 36 hours the numbers were equivalent to 116 per cent. Fat also could be butyrised by heating it with tri-butylin in the presence of a suitable catalyst, such as 1 per cent stannous hydroxide, at 392° to 482° F., the mixture being agitated by allowing an inert gas to bubble through it. Coconut fat containing from 5 to 15 per cent tri-butylin was free of bitterness after 2.5 to 7 hours; while arachis oil with 25 per cent tri-butylin required treatment for 11 hours.

Acetylmethylcarbinol and Diacetyl

van Niel *et al.* (126) studied the relationship of amc and ac_2 to the aroma of butter. Analyses on butter, using 50 g. portions, showed that lots having a typical aroma gave an amc reaction while lots lacking such an aroma did not. A series of lots that had been given definite scores on aroma showed a rather close correlation between a high aroma and the presence of amc . The authors further observed that a dilute aqueous solution of amc had an odor suggesting butter and that ac_2 also had such an odor. However, properly purified amc was odorless. The results suggested that ac_2 is responsible for the flavor of butter. When 0.0002 to 0.0004 per cent ac_2 was added to butter lacking aroma, an unmistakable aroma appeared. The authors concluded that ac_2 is either responsible for the aroma of butter or is the principal component of the aroma material.

The conclusion of Testoni and Ciusa (123) was that the presence of ac_2 in butter is accidental and that ac_2 cannot be considered one of the aromatic principles of butter.

Waser and Mohler (132) noted that even after purification, and in very dilute solution, ac_2 displayed the characteristic odor of butter. Tapernaux (120) stated that the artificial perfumes with an ac_2 base have given good results in creating the artificial aroma of butter in various fatty materials. Development of aroma in natural butter was believed due to the formation of ac_2 by fermentation. Ac_2 was not considered the only substance responsible for development of the natural aroma, but it was believed to be the fundamental base of butter aroma since small amounts possessed a very penetrating odor.

Kay (54) reported that the flavor, and particularly the aroma, of butter is due largely to the presence of ac_2 . In commenting on this idea, Cox (54) stated that he had tested many samples of butter but had never found ac_2 . The compound was not considered present in natural butter.

According to Hammer (40) the odor of ac_2 in high concentrations is pungent and not at all pleasing; however, in low concentrations the aroma is like the aroma of fine butter made from clean-flavored sweet cream with use of culture. Results obtained by Barnicoat (6) indicated that a fraction of a part of ac_2 per million imparts a rich aroma to butter made from un-

ripened cream. Examination of mild-flavored butter showed amc to be present.

Pien *et al.* (91) stated they had good reason to assume that ac_2 is not the cause of aroma in butter even though dilute aqueous solutions have an odor resembling butter and addition of ac_2 to butter without aroma gives an unquestionable aroma.

Virtanen and Tarnanen (129) considered ac_2 to be the substance responsible for the flavor of butter. Davies (23) reported that the compound responsible for most of the flavor of butter is the diketone, ac_2 , which can be produced synthetically or biologically. Butterfat itself was believed to contribute to the flavor, as well as traces of esters, such as ethyl butyrate, caproate and lactate, the curd and the salt. According to Slatter (116) the desirable flavor and aroma of butter are due chiefly to the fat, volatile acids and ac_2 .

Analyses by Pien *et al.* (92) showed that butter high in ac_2 had a very desirable flavor and aroma. However, many samples low in ac_2 also were excellent in flavor and aroma. Results indicated that constituents other than ac_2 are responsible for the desirable flavor and aroma of butter. Pien (90) noted that when ac_2 was absent in butter, the aroma of the butter was weak or there was no aroma.

Mazé (71) stated that the normal lactic acid organisms produce neither amc nor ac_2 and the presence of these compounds in butter proves the existence of an incidental and noxious fermentation. Ac_2 was believed to occur in the blood and, because of this, traces of it were thought to be carried into the milk.

Results of Davies (25, 26) indicated that the flavor and aroma of ripened cream can be closely simulated by working synthetic ac_2 into flavorless butter. However, this procedure gives a somewhat harsh flavor which may be due to the fact that the added ac_2 does not have the same opportunity to distribute itself between the fat and water phases of butter as the ac_2 slowly formed during cream ripening, or it may be due to modification of the effects of ac_2 by other flavoring compounds, such as esters and other volatile organic compounds, which are produced in traces in the cream. The author indicated it is generally agreed that the compound responsible for the aroma and flavor of butter is ac_2 and that it is formed together with somewhat larger amounts of amc.

Experiments by Makar'in (68), designed to test previous theories regarding formation of aroma in butter, indicated that the most important part is played by amc and ac_2 .

Pont (94) noted that it is generally agreed the intensity of flavor and aroma of butter varies with the ac_2 content. Where full development of the butter culture in the cream was permitted, it appeared that the flavoring element existed mainly in the form of amc due to the low oxidation-reduction

potential of the lactic acid fermentation. Besides ac_2 , other flavor contributants were butterfat and traces of esters like ethyl butyrate, caprate and lactate.

According to Langton (59) ac_2 is the cause of the aroma of butter. Davies (27) stated that addition of ac_2 to fats in amounts equal to those found in natural butter gives a harsh, artificial flavor. He suggested that when synthetic ac_2 is added, only about one-tenth of the amount found in butter of equal flavor should be employed. The main argument in favor of the use of synthetic preparations was that non-flavored butter intended for long keeping could be made from sweet cream and the flavor added without detriment to keeping qualities.

Homologs of Diacetyl

According to Waser and Mohler (132) the next higher ketone homolog of ac_2 , triketopentone ($CH_3 \cdot CO \cdot CO \cdot CO \cdot CH_3$), displays a more intense odor than ac_2 . It possesses a penetrating and adhering aroma of ginger bread spice.

Prill *et al.* (97) noted that acetylpropionyl, acetyl-*n*-butyryl, acetyl-*iso*-butyryl, dipropionyl, acetyl-*n*-caproyl and di-*n*-butyryl resembled ac_2 in odor and certain other properties, but methylglyoxal and other alpha keto-aldehydes, as well as the alicyclic and aromatic diketones, differed distinctly from it in odor and also other respects. The various compounds showed similar behaviors in many of the reactions used for determination of ac_2 . Methylglyoxal and glyoxal, however, could be distinguished from alpha diketones by means of the alpha-methylindole test. Certain homologs of ac_2 (acetylpropionyl and dipropionyl), as well as ac_2 , could be determined colorimetrically as diammino-ferrous derivatives of their dioximes. When acetylpropionyl or dipropionyl was worked into salted butter, the effect on the flavor definitely suggested the effect of ac_2 but was less than with an equivalent amount of ac_2 and was less with dipropionyl than with acetylpropionyl. In butter, acetylpropionyl or dipropionyl contents, comparable to the ac_2 contents developed through use of cultures, were well retained over a considerable period at various temperatures. Distillates from ordinary butter cultures gave no evidence of the presence of higher homologs of ac_2 or amc ; methylglyoxal also was absent.

Miscellaneous

Aschan (4) concluded that large quantities of butter are required to obtain the active constituents causing the aroma of butter and that a more profitable attack would be to synthesize the mono- and di-glycerides of the fatty acids which affect the senses of taste and smell.

Tapernaux (120) stated that numerous chemical products have been recommended for production of aroma in butter. Some of these are: Essence

of hazelnuts, essence of bitter almonds (benzoic aldehyde), essence of mirbane (nitro-benzene), coumarin, vanillin, butyric acid and its esters (monobutyrim, *iso*-propyl butyrate, etc.). He noted that none of the above products, either alone or in combination, duplicates the exact odor of butter and suggested that the best technic for developing the aroma of butter in a fatty material is to initiate a natural fermentation which permits development of the aromatic substances *in situ*.

Since lactic acid is odorless it cannot influence the aroma of butter. However, when present in appreciable amounts, it definitely affects the taste.

FACTORS INFLUENCING ACETYLMETHYLCARBINOL AND DIACETYL CONTENTS OF BUTTER

A series of factors related to the manufacturing procedure have an influence on the amc and ac_2 contents of butter and thus, presumably, on the flavor of the butter.

Influence of Strain of Butter Culture

Practical experience indicates that there are wide variations in the butter cultures used in different plants. In some cases cultures have definite defects, lack of flavor being the most common (43; p. 142), but even with normal cultures there are flavor variations (43; p. 102) of significance. These variations may not be interpreted the same by different persons, one culture in a series being preferred by one person and another by some one else. Because of variations in a culture from day to day, due to the acidities developed and other factors, the cultures in a series may not be rated the same on different days. In general, an experienced person can very accurately judge the value of a particular culture for development of flavor in butter.

Watts (131) compared nine commercial cultures and found there was no particular culture which was superior to the others as far as flavor of the butter was concerned. Knudsen (57) noted a close correlation between the aroma of butter and the aroma of the culture used.

Slatter and Hammer (117) reported an increase in the $\text{amc} + \text{ac}_2$ contents of salted and unsalted butter with an increase in the amount of culture employed; this suggested that the flavoring materials in the butter come largely from the culture rather than from changes in the cream during holding.

Krenn (58) added 5 per cent of three cultures containing 0.595, 0.71 and 3.98 mg. ac_2 per l., respectively, to three lots of cream. The cream was ripened and before churning contained 1.85, 1.012 and 2.42 mg. ac_2 per l., respectively. The amounts of ac_2 contributed by the cultures were 0.02975, 0.0357 and 0.199 mg., respectively, while the amounts formed in the cream were 1.82, 0.9763 and 2.22 mg., respectively.

Results obtained by Prill and Hammer (98) showed that, when culture was added to sweet cream, the cream-culture mixture contained approximately the amount of amc contributed by the cream and the culture, whereas the ac_2 content was regularly higher than the calculated amount. The authors suggested that incorporation of air during the mixing presumably made it possible for the organisms to effect considerable oxidation. After holding overnight at 36° to 44° F., the ac_2 had increased further, often more than doubling, and the amc also showed some increase but the percentage increase was not as great as with ac_2 . No close relationship was noted between the ac_2 contents of the cultures and of the finished butter with churnings made by adding the cultures to sweet cream. Results obtained with culture and sour cream showed that more ac_2 was contributed to the cream-culture mixture by the neutralized and pasteurized cream than by the culture; amc was contributed in comparable amounts from the two sources. However, the culture supplied active organisms which made possible a further accumulation of the two compounds. Changes occurring in the ac_2 and amc contents were similar to those obtained with mixtures of sweet cream and culture.

In the trials of Hedrick and Hammer (45) on the ripening of sweet and sour cream for unsalted butter, the amc and ac_2 contents developed by different cultures varied rather widely in some instances and were rather uniform in others. When the same cultures were used in several trials they did not always rank the same on the basis of production of amc or ac_2 , and the variations were not explainable on the basis of the slight differences in acidities of the ripened cream. Also, with the same cultures being used in several trials, each of them sometimes showed relatively high values and sometimes relatively low values.

Influence of Acidity of Cream

Investigations have clearly established that in a butter culture relatively large amounts of amc and ac_2 are only produced when considerable acid has developed (43; p. 114). This general relationship also applies in the ripening of cream. Because of it also, the amc and ac_2 contents of the original cream are significant only when the cream has developed some acid.

Tapernaux (120) stated that during the course of cream ripening acid develops and there appears a particular aroma due to the lactic acid organisms. He detected ac_2 in ripened cream; it was most evident when the ripening was not preceded by pasteurization. According to Kay (54) acid formation in cream is essential for aroma production since sweet cream does not contain amc. In the experiments of Barnicoat (6) butter made from cream ripened with culture showed amc + ac_2 contents which paralleled the rise in acid during the ripening.

Michaelian and Hammer (73) found the amounts of amc + ac_2 in sour cream delivered to butter plants was relatively high. They stated that this

was to be expected because of the wide distribution of flavor organisms and the favorable conditions provided by the acid cream for production of $\text{amc} + \text{ac}_2$.

Barnicoat (6) indicated that there is little $\text{amc} + \text{ac}_2$ developed when cream is ripened to the small extent common in the manufacture of mild-flavored butter. Most of the flavor-producing substances present in butter made from slightly ripened cream are not developed during the ripening but are added with the culture. This was demonstrated by experiments in which butter made from cream containing only 0.5 per cent culture, and in which no acid was developed, had a distinct aroma.

In the trials of Bouska (10) cream which had soured spontaneously for a moderate period, and which was churned without culture, produced butter with aroma. However, cream which had soured so long that amc and ac_2 had disappeared did not produce aromatic butter unless culture was added. Bungler (16) and also Mohr and Wellm (79) noted that butter made from sweet cream had much lower amc and ac_2 contents than butter made from sour cream. Makar'in (67) reported that the amc and ac_2 contents increased sharply during souring of cream; the same was true during souring of skimmilk.

Davies (24) stated that the rate of formation of ac_2 in cream can best be measured by calculating the ratio of ac_2 to acid (ac_2 as ppm. and acid as per cent lactic). When cream was ripened for 3 hours at 89.6°F . with a cheese culture the ratio was 3, with a butter culture it was 6 and with a *S. cremoris* culture it was 24; acidities were 0.25, 0.24 and 0.20 per cent, respectively. Incubating for longer periods showed that the ac_2 content of the *S. cremoris* culture increased more rapidly than that of the other two cultures with less increase in acid. The total ac_2 and amc in each culture increased roughly with acid, but at different rates. In 6 hours the ac_2 to amc ratio was 1:40 with the cheese culture, 1:20 with the butter culture and 1:18 with the *S. cremoris* culture. Davies (25) also noted that the most desirable property of a culture is that of forming the largest amount of ac_2 at a low acidity since in higher acid ranges it is the amc which is formed in largest amount; in general, at low acidities the ratio of ac_2 to amc is greater than at higher acidities.

According to Davies (25) when a culture is used to acidify cream, the titrable acidities of the cream which correspond to flavorless butter, mild-flavored butter, medium-flavored butter and full-flavored butter are 0.10 to 0.15, 0.15 to 0.25, 0.25 to 0.40 and 0.40 to 0.75 per cent, respectively. These figures apply to cream containing about 35 per cent fat. The cream serum acidities would be 0.13 to 0.20, 0.20 to 0.30, 0.30 to 0.50 and above 0.50 per cent, respectively. Various factors other than acidity which influence the degree of flavor development were listed as: (a) Distribution of the various types of lactic acid bacteria in the culture; (b) their virility under com-

petitive conditions in cream; (c) citric acid and oxygen contents of the cream; and (d) degree of biochemical and oxidation reactions occurring in the ensuing butter. Later, Davies (27) reported that the following titrable acidities in cream will ensure the various degrees of butter flavor: Mild flavor, 0.20 to 0.25 per cent; medium flavor, 0.25 to 0.35 per cent; and full flavor, 0.35 to 0.55 per cent. The corresponding ac_2 contents of lots of butter of ascending degrees of flavor were: Mild flavor, 0.5 to 1.5 ppm.; medium flavor, 1.5 to 2.5 ppm.; and full flavor, 2.5 to 4.0 ppm. These conditions referred to cream of 30 to 38 per cent fat, pasteurized by the flash method at 180° to 190° F., cooled and ripened at 65° to 70° F. to the desired acidity with butter culture. The author stated that if depth of flavor of butter is of importance, it is necessary to produce as much ac_2 as possible during the initial stages of cream ripening. That is, the flavor organisms must produce aroma before *S. lactis*, which produces acid only, depletes the cream of its dissolved oxygen. The ratio of ac_2 to amc was believed to depend on oxidation-reduction reactions occurring in the cream and these are governed, to an appreciable extent, by the oxygen tension in the cream.

Results of laboratory trials by Hedrick and Hammer (45) on sweet cream ripened to various acidities at 50° to 70° F. showed that as the acidity of the cream increased the ac_2 content increased in 29 (81%) of the 36 trials and the amc content in 32 trials (89%). Most of the increases were significant, but some of them were very small. Both ac_2 and amc increases were small in the pH range 6.5 to 5.5. In the remaining trials the ac_2 and amc contents showed changes other than regular increases as the acidity of the cream increased. Ac_2 contents of six lots of sweet cream with added culture held for a normal ripening period at 32° F. ranged from 0.12 to 0.28 ppm. (average 0.22 ppm.); the amc contents varied from 3.5 to 16.5 ppm. (average 10.6 ppm.). Three trials were carried out with neutralized sour cream ripened to various acidities at 58° to 62° F. Three acidities were used in each of two trials and four acidities were employed in one trial; in each trial a portion of the cream-culture mixture was held at 32° F. for the normal ripening period. An increase in acidity resulted in an increase in ac_2 and also in amc in each case. Increases in ac_2 were conspicuous and in two of the trials increases in amc were large. The ac_2 and amc contents of the three lots of neutralized sour cream with added culture held at 32° F. generally were higher than those of sweet cream with added culture, the average ac_2 content being 0.42 ppm. (from 0.35 to 0.55 ppm.) and the average amc content 22.1 ppm. (from 20.9 to 23.3 ppm.). This relationship is explained by development of the two compounds during the souring of the cream.

In ten trials on a semi-commercial scale Hedrick and Hammer (45) ripened sweet cream (seven trials) and sour neutralized cream (three trials) to various acidities at 50° to 55° F. Three acidities were employed in three of the trials with sweet cream and two acidities were used in the

remaining trials. In two trials with sour cream, ac_2 and amc determinations were made just after pasteurization and again after addition of culture. With an increase in acid there was an increase in ac_2 in nine trials (90%) and in amc in seven trials (70%). Some of the increases were small. Ac_2 decreased in one trial (10%), the drop being from 1.88 to 1.49 ppm. with an increase in acid from 0.37 to 0.49 per cent. Amc decreased in three trials (30%), in one instance after a striking increase; two of the trials involved sour cream in which the citric acid may have been completely fermented. In general, as the ac_2 increased during the ripening of cream the amc also increased, but in some instances there was an increase in ac_2 and a decrease in amc. Commonly, increases in ac_2 and amc were in the same general proportion, but again there were exceptions.

The effect of ripening acidity on the score of unsalted butter was studied by Hedrick and Hammer (45). When the butter was held 1 week at 36° to 40° F., ripening cream to the higher of the two acidities used in each trial gave butter which scored 0.25 to 0.75 of a point higher than ripening to the lower acidity in five (71%) of the seven trials; there was no difference in score in one trial (14%) in which the acidities of the two lots of cream differed only 0.04 per cent; and in one trial (14%) the butter from the higher acid cream scored 0.25 of a point lower but the difference in the cream acidities was small, although both were relatively high (0.48 and 0.51%). After 1 month at 36° to 40° F., the butter made from the higher acid cream scored 0.25 to 0.5 of a point higher in three trials (43%), no difference in score was noted in two trials (29%) and the butter from the higher acid cream scored 0.5 of a point lower in two trials (29%). After 6 months at -10° to 0° F., ripening to the higher acidity gave butter which scored from 0.25 to 0.75 of a point higher in all trials.

Influence of Temperature of Ripening

Various investigations have dealt with the effect of ripening temperature on production of amc and ac_2 in butter cultures and similar studies have been carried out in connection with cream ripening.

Bunger (16) reported that cream allowed to sour at 62.6° F. had the highest content of amc and ac_2 .

Wiley (135) inoculated sweet cream with 10 per cent culture and incubated it at 80° F. When ripened to pH 5, the cream contained about 0.8 ppm. ac_2 and 18 ppm. amc. Pasteurized sweet cream ripened with culture to pH 5 and then held 24 hours at 48° F. contained about 4.0 ppm. ac_2 and 90 ppm. amc.

Mohr and Wellm (79) noted that the ac_2 content of butter varied with the ripening temperature of the cream. Temperatures of 62.6° F., 50° F. and a combination of 62.6° and 50° F. were studied. Butter made from cream ripened at 62.6° F. contained 0.36 mg. ac_2 per kg. and 2.76 mg.

ame + ac₂; these were the largest amounts found. According to Mazé (71) the most favorable temperature for development of flavor and aroma substances in cream is 57.2° to 60.8° F.

Fabricius and Hammer (33) determined the amounts of ame + ac₂ in cream held overnight at 28° to 36° F. after adding 8 per cent culture, in cream held overnight at 42° to 51° F. after adding 8 per cent culture, and in cream ripened 1 hour at 70° F. and then cooled and held at 28° to 36° F. after adding 8 per cent culture. Results showed that the unripened cream held at 42° to 51° F. usually was higher in ame + ac₂ than the unripened cream held at 28° to 36° F. and contained about the same amounts as the ripened cream held at 28° to 36° F. Amounts of ame + ac₂ in cream held cold after adding culture 16 hours before churning and in cream to which culture was added at churning also were compared. The higher ame + ac₂ contents were found with addition of culture 16 hours before churning. Another series of comparisons involved cream held at a low temperature for 3, 6, or 16 hours after adding culture; there were both increases and decreases in ame + ac₂ contents as a result of the holding. Effects of holding for 64, 70, 88 or 112 hours also were variable. Immediately after adding culture the ame + ac₂ contents of the different portions in a trial were essentially the same.

Laboratory tests on the effect of ripening temperature on the ac₂ and ame contents of cream were made by Hedrick and Hammer (45). In 18 trials sweet cream was ripened at 50°, 60° and 70° F. Ripening periods varied from 5 to 45 hours, depending on the temperature and the degree of acid desired. Ac₂ and ame determinations were made soon after the desired acidity was reached. The highest ac₂ content developed at 50° F. and the lowest at 70° F. in 14 of the trials (78%); the same relationship occurred with ame in 11 trials (61%). Both ac₂ and ame production were highest at 50° F. and lowest at 60° F. in two trials (11%). The highest ac₂ content was produced at 60° F. and the lowest at 70° F. in one trial (6%); the same relationship occurred with ame in five trials (28%). Ac₂ production was highest at 70° F. and lowest at 60° F. in one trial (6%). Variations in ame production at the three temperatures were small and definitely less on a percentage basis than variations in ac₂ production. Four trials were carried out on a semi-commercial scale; three involved sweet cream and one involved neutralized sour cream. Ac₂ production in cream ripened at 62° F. was larger than in cream ripened at 70° F. in two trials (50%) and smaller in two trials (50%). Ame production showed similar variations. Differences in production of ac₂ and ame in a comparison commonly were small. In three trials, 0.1 per cent citric acid was added to the cream and the cream agitated during ripening overnight to an acidity of 0.45 per cent. Ac₂ and ame production was much greater in each trial than in the cream ripened at either 62° or 70° F.

Effect of ripening temperature on the score of the resulting unsalted butter was studied by Hedrick and Hammer (45). With butter held 1 week at 36° to 40° F., ripening of the cream at 62° F., rather than 70° F., gave a score increase of 0.25 to 0.75 of a point in two trials (50%), no difference in score in one trial (25%) and a score decrease of 1 point in one trial (25%). With butter held 1 month at 36° to 40° F., ripening at 62° F. gave a score increase of 0.5 to 0.75 of a point in each of three trials (100%). With butter held 6 months at -10° to 0° F., ripening at 62° F. gave a score increase of 0.5 of a point in each of two trials (50%) and a score decrease of 0.75 and 1.0 point in two trials (50%).

Hoecker and Hammer (47) found that on holding cream plus culture 16 hours at 40° F., the ac_2 and amc contents increased when butter culture, *S. diacetylactis* or *S. citrophilus* was used; decreases often occurred with *S. citrovorus* or *S. paracitrovorus*; usually little or no change occurred with an unidentified organism or *S. aromaticus*.

Influence of Fat Content of Cream

Variations in the fat contents of different lots of cream are large enough to be of importance from the standpoint of cream ripening and butter manufacture. With a high fat content the serum content is small, and a certain percentage of butter culture, based on the cream, has a greater effect in lowering the pH, etc., than when the fat content is low and the serum content correspondingly high. On the other hand, with a high serum content the percentage of citric acid in the cream is larger.

Orla-Jensen (85) stated that since the lactic acid bacteria do not act on butterfat they must produce the characteristic butter aroma and acid from the other constituents of the milk; therefore, the richer the cream, the less aroma will be formed therein.

Barnicoat (7) investigated the effect of the growth medium on development of ac_2 and amc . Experiments made with milk and cream steamed for 30 to 40 minutes, allowed to cool and inoculated with 0.5 per cent culture showed that there was no essential difference, with due allowance for the fat content of the cream, in the action of culture in skimmilk, whole milk and cream.

Ac_2 and amc contents of butter churned from whole milk and from cream of varying fat contents were determined by Mohr and Wellm (79). Butter made from 40 per cent cream contained the most ac_2 , while butter made from 20 per cent cream and that made from whole milk were about equal in ac_2 contents. Amc contents of the lots of butter increased with increasing fat contents of the cream. Bungler (16) noted that on churning sour whole milk, 20 per cent cream and 40 per cent cream, the ac_2 content was highest in the butter from the 40 per cent cream; butter made from the whole milk and that made from 20 per cent cream contained practically the same amounts of ac_2 .

Bogdanow (9) recommended an increase in the fat content of the cream in order to obtain increased aroma. He stated that aroma is absorbed by the fat.

Influence of Addition of Citric Acid to Cream

Since citric acid is the important source of flavor materials in butter cultures (43; p. 116) and the amount in milk is comparatively small (43; p. 102), this acid or its sodium salt sometimes is added to milk intended for culture. Such an addition also would be expected to have an effect in the ripening of cream.

Preliminary experiments from the Union of South Africa (3) indicated that cream churned with added citric acid gives butter of better flavor than that from untreated cream. Orla-Jensen (85) reported that autolysed yeast extract added to cream did not afford a sure means of increasing the aroma of butter but addition of citric acid gave more promising results. Templeton (121) found that addition of citric acid or sodium citrate to butter culture, to the cream used for butter or to both gave butter with a more pronounced flavor and aroma. Addition of 0.2 per cent citric acid, or its equivalent of sodium citrate, to the culture alone had a slight effect. The effect was more pronounced when the same proportion of citric acid or citrate was added to the cream.

Khubchandani (55) noted that addition of citric acid or sodium citrate to cream resulted in butter with increased aroma but poorer keeping qualities. Hunziker (48; p. 364) concluded that, in the absence of more favorable experimental data, addition of citric acid to cream appears to be of doubtful merit; he suggested avoiding the expense until more convincing knowledge of the merits of the addition is available.

Experiments involving addition of citric acid to cream at the time of inoculating with culture were reported by Hedrick and Hammer (45). In 14 laboratory trials sweet cream was ripened with and without added citric acid to about the same acidity at temperatures from 54° to 60° F.; 0.05 per cent acid was added except in one trial in which 0.10 per cent was used. Addition of citric acid gave a higher ac_2 content in the cream in all the trials (100%) and a higher ame content in 12 trials (86%). With both ac_2 and ame some of the increases were very small. In eight trials neutralized sour cream was ripened with and without citric acid to about the same acidity at temperatures from 58° to 63° F. In six trials (75%) added citric acid resulted in an increase in the ac_2 content. In the four trials involving three amounts of acid, the increases were roughly in proportion to the amount added; in one trial (12.5%) 0.05 and 0.10 per cent added citric acid did not increase the ac_2 content but 0.15 per cent did, and in one trial (12.5%) 0.10 and 0.15 per cent citric acid gave the same ac_2 content. Added citric acid regularly increased the ame content and, in the trials in which three amounts were used, the increases were roughly in proportion to the amount

added. Data were obtained on seven semi-commercial trials in which cream was ripened with and without added citric acid (0.05%) to about the same acidity at temperatures from 50° to 54° F. Added citric acid increased the ac_2 content in six comparisons (86%); the increases varied widely, with three of them being 0.20 ppm. or less. The one decrease in ac_2 content was very small, being 0.13 ppm. Added citric acid increased the amc content in three comparisons (43%), the increases ranging from 12.8 to 34.8 ppm. In one trial (14%) the amc content was the same with and without added citric acid. In the three comparisons (43%) in which addition of the acid resulted in a decrease in amc content, the decreases ranged from 4.0 to 15.8 ppm.

Hedrick and Hammer (45) studied the effect of adding 0.05 to 0.10 per cent citric acid to cream on the score of the resulting unsalted butter. With butter held 1 week at 36° to 40° F., addition of citric acid increased the score 0.25 to 0.50 of a point in four trials (57%) and there was no difference in score in three trials (43%). With holding 1 month at 36° to 40° F. the addition gave a score improvement of 0.25 to 0.75 of a point in six trials (86%) and no difference in score in one trial (14%). With holding 6 months at -10° to 0° F. the addition resulted in an increase in score of 0.5 to 0.75 of a point in six trials (86%) and a score decrease of 0.5 of a point in one trial (14%).

Influence of Aeration of Cream

The oxygen supply is a factor in the production of amc and ac_2 in butter cultures (43; p. 118) and, accordingly, should be important in flavor development in cream. However, aeration may have an undesirable influence on the flavor of some of the milk constituents because of their tendency to oxidize.

According to Orla-Jensen (85) aeration of cream may possibly promote production of aroma; it increased the volatile acidity of cream. Davies (23) noted that most of the ac_2 in cream is formed while there is considerable atmospheric oxygen in solution.

Virtanen and Tarnanen (129) considered that the fact ac_2 is formed only when oxygen acts as the hydrogen acceptor should be considered in butter manufacture. They suggested that cream be ripened under as aerobic conditions as possible and that churning be carried out so as to ensure a maximum content of air in the butter. (See also 128.) Virtanen (128) found that passing sterile air into cream during ripening had a very favorable effect on the aroma of fresh butter, the ac_2 content being increased considerably. Makar'in (67) stated that the upper layers of souring cream are richer in ac_2 and amc than the lower layers.

Davies (24) reported that formation of amc was favored by a low oxygen content. Later, he (25) indicated that when a culture was added to cream,

the important factor was the rate at which the aroma-producing organisms proliferated before the true lactic acid bacteria induced a low oxygen content. Ac_2 was found in cream in greatest amounts when there still was some free oxygen present.

It was noted by Prill and Hammer (98) that when sweet cream and aerated-pressure (p. 89) or modified aerated-pressure (p. 88) culture were mixed, analyses did not indicate an increase in ac_2 during the mixing but sometimes suggested a slight decrease, especially in the case of aerated-pressure culture. During the holding of the cream-culture mixtures overnight and during churning, the organisms usually produced additional ac_2 and amc ; although in most cases the actual increment in ac_2 was as great (or greater) than when regular butter culture was used, the percentage increase was not because of the relatively large amount of ac_2 originally present. It appeared that if the cream had been churned soon after addition of culture, aerated-pressure or modified aerated-pressure culture probably would show a greater practical advantage over regular butter culture.

Laboratory trials on the effect of agitation during the ripening of cream on the ac_2 and amc contents were reported by Hedrick and Hammer (45). In nine trials (seven with sweet cream and two with neutralized sour cream) cream was ripened with and without agitation at 60°, 63° or 70° F. At 60° or 63° F. the cream was agitated three times during the ripening while at 70° F. it was agitated every hour for 3 or 4 hours. In each trial the ac_2 and amc contents of the agitated cream were much higher than those of the unagitated control. Essentially the same acidity developed with and without agitation. Effect of agitation with added citric acid was studied in six trials (four with sweet cream and two with neutralized sour cream); 0.10 per cent citric acid was used and the ripening temperature was 60°, 63° or 70° F. As without addition of citric acid, agitation greatly increased both the ac_2 and amc contents but had no appreciable effect on acid development. Five trials were carried out on a semi-commercial scale (three with sweet cream and two with neutralized sour cream). Again the ac_2 and amc contents were greatly increased by the agitation whereas the acid development was not significantly influenced.

In a study of the effect of agitation during ripening on the score of the resulting unsalted butter after 1 week at 36° to 40° F., Hedrick and Hammer (45) found that agitation of the cream gave a score increase of 0.25 to 0.75 of a point in three (60%) of the five trials and no difference in score in two trials (40%). With holding of the butter 1 month at 36° to 40° F., agitation gave a score increase of 0.5 to 0.75 of a point in two trials (40%), no difference in score in two trials (40%) and a score decrease of 0.25 of a point in one trial (20%); with holding 6 months at -10° to 0° F., agitation resulted in a score increase in four trials (80%) and no difference in one trial (20%).

Hoecker and Hammer (47) reported that immediately after mixing the ac_2 contents of cream with added butter culture were sometimes higher and sometimes lower than the theoretical amounts calculated from the ac_2 contents of the cream and culture; in most trials the amc contents were about the same as the theoretical values but in some instances were higher.

Influence of Neutralization and Pasteurization of Cream

Neutralization and pasteurization of cream constitute the primary treatments of much of the cream intended for butter, neutralization now being employed even with some of the sweet cream used for butter. In the investigations of the effect of these processes on the amc and ac_2 contents of cream, there has been some variation in the results, probably because of variations in the types of cream used and in the neutralization and pasteurization procedures.

Barnicoat (7) found that neutralization and pasteurization of cream caused no notable decrease in ac_2 but a distinct decrease in amc. Destruction of amc was correlated with the neutralization point of the cream and was similar with the two types of pasteurizers used; it was thought to have some bearing on the opinion, frequently expressed, that butter made from over-neutralized cream often is deficient in flavor.

Davies (24) noted that cream which had developed certain amounts of amc and ac_2 did not yield butter having flavor or aroma when the cream was neutralized to 0.10 to 0.15 per cent acid and flash pasteurized. Disappearance of amc and ac_2 from the cream was explained on the basis of volatility of the aroma constituents and absence of oxidation of amc in the butter. Examination of many samples of neutralized cream butter after 6 months in storage revealed no trace of ac_2 or amc + ac_2 by the usual analytical methods. Later Davies (26) stated that butter made from neutralized cream possesses no butter flavor; if flavor is required, the pasteurized and cooled cream is ripened by addition of culture to different acidities, depending on the degree of flavor desired.

According to Prill and Hammer (98) neutralized and pasteurized cream frequently had a lower ac_2 content than the raw cream. With cream having an original acidity of 0.5 to 0.6 per cent, it was found that after preliminary neutralization to 0.25 per cent acid, or during the first part of the heating, the ac_2 content fell to a very low value and the amc content commonly decreased slightly. The compounds may have been reduced to 2,3-butylene glycol. When the cream had been heated to pasteurizing temperature, and also in subsequent examinations, the ac_2 contents showed very significant increases. The amc content showed an increase when the cream had reached pasteurizing temperature and then remained fairly constant in the subsequent examinations. The authors stated that the increase in ac_2 may possibly have been caused by non-biological oxidation but the increase in

ame through non-biological reactions seemed improbable. Net result of the cream processing was a decrease in ac_2 content and relatively little change in ame content.

Influence of Certain Metallic Salts

Mohr and Arbes (77) noted that when milk or cream was treated with certain metallic salts and then soured and stored for approximately 20 hours, or allowed to sour during such storage, the ac_2 content was increased over that of the control lot. The salts tested, in order of increasing effectiveness, were cupric chloride, manganese chloride, ferric chloride and a combination of all three; they were added in amounts of 0.5 to 2.0 mg. per l. of milk or cream. Addition of the salts to milk or cream immediately before determining the ac_2 was without effect on the results.

Influence of Churning

Because of the agitation of the cream and the butter granules in the presence of air, churning would be expected to have an effect on the ac_2 contents of the cream and the resulting butter and perhaps on the ame contents.

Tapernaux (120) stated that the butter formed by agglomeration of fat globules from the cream during churning seems to retain the aromatic substances, and the aroma of butter continues to increase in intensity throughout the days which follow manufacture; the butter also contains fermentable substances and ferments. According to Davies (22) ac_2 probably is not formed until butter is churned and stored. Bungler (16) noted that the ac_2 content of cream increased during churning.

Mazé (71) considered that churning must be regarded as an additional aerobic fermentation, its object being to oxidize the reductive products of the anaerobic lactic acid fermentation and to produce the flavor characteristic of fresh, first-quality butter. Results obtained by Mohr and Wellm (79) indicated that the largest contents of ac_2 and ame were obtained in fresh butter at a churning temperature of 57.2° F.

Virtanen (128) stated that churning of cream in a churn filled with some indifferent gas is obviously not suited to production of butter with a rich aroma. He suggested that churning should be carried out in such manner as to ensure a maximum content of air in the butter since this promotes formation of ame and finally ac_2 .

The analyses of Prill and Hammer (98) showed an increase in ac_2 content during churning. The authors noted that the ame content would not be expected to change greatly in the short period involved.

Influence of Washing Butter with Water

Because of the solubility of ame and ac_2 in water, the washing of butter would be expected to remove some of these materials from it.

In 1893, Conn (20) noted that the flavor of butter was very much more prominent without thorough washing than with it. If the butter was washed long enough, all of the aroma could be washed away.

Leitch (60) indicated that much of the aroma of butter is lost during the washing. He recommended washing butter with serum prepared by fermenting pasteurized skim milk with culture, removing the curd and filtering the liquid through sterile linen. Testoni and Ciusa (123) found that when butter was washed four times it no longer contained ac_2 ; when washed only twice it contained the compound.

According to results obtained by Barnicoat (6) no extraction of $amc + ac_2$ appeared to take place during the washing of butter. Later he (7) reported the loss of $amc + ac_2$ by the washing of butter was relatively unimportant.

Krenn (58) noted that butter wash water contained considerable ac_2 . Bungler (16) removed the greater part of the amc and ac_2 from butter granules by washing them three times. Makar'in (67) reported that washing butter greatly reduced the ac_2 content. In the analyses of Mohr and Wellm (79) unwashed butter contained 1.69 mg. ac_2 per kg. and 9.30 mg. $amc + ac_2$ whereas washed butter contained 0.86 mg. ac_2 and 3.78 mg. $amc + ac_2$. According to Pont (94) approximately four-fifths of the $amc + ac_2$ originally present is removed by the buttermilk and the washing of the butter.

Mohr and Arbes (77) washed butter with water containing 0.5 to 1.5 mg. per l. of various salts of iron, manganese and copper; they concluded that the salts had no effect on the ac_2 content of the butter.

Prill and Hammer (98) analyzed several samples of butter wash water and found only a fraction of a mg. of $amc + ac_2$ per kg. They stated that while the total $amc + ac_2$ in wash water may be significant, compared to the amount present in the butter, it is difficult to differentiate between material which comes from the film of buttermilk remaining between the butter granules and that which might possibly be removed from the granules themselves. If amc and ac_2 are both held entirely in the serum droplets in the same manner, they should be retained in the butter in the same proportion as they exist in the churning mixture at the time the butter is formed, and any possible washing out of the compounds should also be in this proportion.

Partition of Acetylmethylcarbinol and Diacetyl During Churning

When cream is churned, some of the amc and ac_2 goes into the butter and some into the buttermilk. This general relationship has led to various studies on the partition of the compounds during churning. Presumably, from the standpoint of butter flavor, it would be better if all the amc and ac_2 present in the cream were retained in the butter.

Comparative amounts of acetylmethylcarbinol and diacetyl in cream and butter. Barnicoat (6) calculated that about one-fifth of the $amc + ac_2$

in cream is carried into the butter. Michaelian and Hammer (73) noted that butter regularly contained much less $\text{ame} + \text{ac}_2$ than the cream from which it was churned. With four lots of butter the $\text{ame} + \text{ac}_2$ was not measurable with the gravimetric method then commonly in use but with the other 52 lots in the series the ratios of $\text{ame} + \text{ac}_2$ contents of the cream to those of the butter ranged from 1:0.032 to 1:0.218. The ratios were not correlated with the amount of $\text{ame} + \text{ac}_2$ in the cream or the acidity of the cream.

Results obtained by Mohler and Herzfeld (76) indicated that cream from which the butter was churned contained about three times more ac_2 than the resulting butter. Davies (23) reported that only about one-fifth of the $\text{ame} + \text{ac}_2$ in cream appeared in the butter and about one-fourth of the ac_2 . Well ripened cream (0.6 per cent acid) containing 5 to 10 ppm. ac_2 and 100 to 250 ppm. ame yielded butter containing 1 to 2 ppm. ac_2 .

According to Barnicoat (7) the percentage of ac_2 retained by butter varies from 0 to 33 per cent, the extremes occurring with neutralized creams. Average retention in the butter was 15 per cent of the amount originally in the cream. The ratio of ac_2 in butter to ac_2 in cream (30 to 40 per cent fat) was 0.4. The ratio of ac_2 to ame in "mild starter" butter examined 1 day after manufacture was greater than in cream; the average ratio of ac_2 to ame in 11 lots of cream was 0.07 while in the butter the ratio was 0.11.

Brioux and Jouis (14) found that butter contained less ac_2 than the cream from which it was churned. Bungler (16) noted that from one-fourth to one-sixth of the ac_2 and from one-fifteenth to one-sixteenth of the ame in the cream was present in the resulting butter.

Barnicoat (7) reported that when ame , as the synthetic compound, was added to pasteurized cream there was, in the absence of butter culture, a loss before churning. Results obtained on 12 churnings showed that from 7 to 22 per cent (average 14%) of the ame in the cream was retained in the butter.

Davies (24, 25, 26) noted that the ratio of ac_2 in cream to that in butter was approximately 4:1; the ratio of $\text{ame} + \text{ac}_2$ in cream to that in butter was between 4:1 and 5:1.

Analyses made by Mohr and Wellm (79) on washed and worked butter showed that the butter had only one-fourth to one-sixth of the ac_2 content of the cream and from one-fifteenth to one-sixteenth of the ame content.

Krenn (58) determined the amounts of ac_2 in butter obtained from cream which contained 1.012 and 2.42 mg. ac_2 per l., respectively. The butter contained 0.357 and 0.00 mg. ac_2 per kg., respectively. Krenn concluded that only a small part of the ac_2 formed during the ripening of cream is present in the resulting butter. Brioux and Jouis (14) found that cream containing 95.2 mg. ame per kg. yielded butter containing 42.4 mg.; the same cream contained 1.92 mg. ac_2 and the resulting butter 1.50 mg. ac_2 .

According to Davies (25) the amount of ac_2 in butter is roughly 10 per cent of that in the cream. The amount in the finished butter was considered to be influenced by the amount of washing and the fraction of the moisture accounted for by the buttermilk droplets which, in turn, depends on the degree of working. The author further stated (27) that the distribution of ac_2 in butter is roughly proportional to the ratio of butter serum to buttermilk.

Results of Hoecker and Hammer (46) showed that only small percentages of the amc and ac_2 in a cream-culture mixture were retained in the butter, the remainder being in the buttermilk; the percentage retention was essentially the same with various cultures, although with each culture there was considerable variation from one churning to another.

Hedrick and Hammer (45) reported that with 74 semi-commercial churnings of unsalted butter, the minimum, maximum and average ratios of the ac_2 contents of the ripened cream and of the corresponding butter were 1:0.142, 1:0.709 and 1:0.352, respectively; the corresponding ratios for amc were 1:0.059, 1:0.683 and 1:0.217, respectively. The investigators also noted that the ac_2 and amc contents of a series of lots of butter sometimes did not follow the same order as the contents of the lots of cream from which the butter was churned. The irregularities were of all possible types. In some cases the ac_2 or amc content of a lot of butter was lower than that of an earlier lot in the series, although the content of the cream was higher. In other cases a value for ac_2 or amc was lower than the values on the lots preceding and following it in the series, even when there was no drop in the value for the cream. Still other types of variations occurred. The ac_2 contents of the semi-commercial churnings of butter ranged from 0.16 to 1.46 ppm., while the amc contents varied from 2.9 to 24.0 ppm. The highest values for both ac_2 and amc were obtained on a lot of butter made from cream ripened with agitation after addition of 0.1 per cent citric acid.

Hoecker and Hammer (47) stated that, in butter made with butter culture, amc and ac_2 are derived largely from the culture added to the cream and from fermentation of citric acid during holding or ripening of the cream.

Comparative amounts of acetylmethylcarbinol and diacetyl in cream and buttermilk. In 1893, Conn (20) noted that, after churning, nearly all the flavor produced during the ripening of cream was contained in the buttermilk. Butter aroma, therefore, was considered due to changes in some constituent of the cream other than fat.

Hammer (40, 41) stated that, when cream was churned, much of the amc and ac_2 in it was carried into the buttermilk so that the buttermilk contained more amc and ac_2 than the cream. Michaelian and Hammer (73) found that fresh buttermilk regularly contained more $amc + ac_2$ than the cream from

which it was obtained. The ratios of $\text{ame} + \text{ac}_2$ in the cream to that in the buttermilk ranged from 1:2.0 to 1:3.1. In a series of churnings involving cream of various acidities, the ratios ranged from 1:1.1 to 1:2.1.

According to Davies (23) aeration of cream during churning, and the consequent readjustment of the ratio of ac_2 to ame in the buttermilk, causes the buttermilk to contain more ac_2 than ripened cream. An increase in the acidity of the buttermilk resulted in a decrease in the ac_2 content.

Bunger (16) found that buttermilk contained from 2.4 to 4.0 times more ac_2 than the sour cream before churning. Results obtained by Davies (24) indicated that the ratio of ac_2 in buttermilk to that in the original cream varies from 2:1 to 3:1, depending on the fat content of the cream and the ratio of ac_2 to ame in the cream.

Krenn (58) determined the amount of ac_2 in buttermilk obtained from cream which contained 1.012 and 2.42 mg. ac_2 per l., respectively, before churning. The buttermilk contained 1.250 and 4.098 mg. per l., respectively. Buttermilk having an initial ac_2 content of 4.59 mg. per l. showed 4.53 mg. after holding 8 hours and 4.65 mg. after holding 24 hours. This increase was believed due to the lactic acid and aroma-forming bacteria which were present in large numbers.

Makar'in (67) noted that the ac_2 content of buttermilk was greater than that of the cream before churning. Results obtained by Mohr and Wellm (79) showed that buttermilk contained 2.4 to 4.0 times as much ac_2 as the cream before churning. Brioux and Jouis (14) stated that after churning the ac_2 and ame in fermented cream were contained largely in the buttermilk. Fermented cream containing 95.2 mg. ame per kg. yielded buttermilk containing 195.3 mg.; the same cream contained 1.92 mg. ac_2 and the resulting buttermilk contained 3.10 mg.

Comparative amounts of acetylmethylcarbinol and diacetyl in butter and buttermilk. van Niel *et al.* (126) found ac_2 in a sample of buttermilk having an exceptionally fine aroma and a high ame content. According to Hammer (41) there is a concentration of ame and ac_2 in the buttermilk rather than in the butter.

In order to check the accuracy of his investigation, Krenn (58) attempted an ac_2 balance. In one trial 10 kg. of cream (21% fat) produced 2.42 kg. of butter and 7.58 l. of buttermilk. The cream (9.88 l.) contained 10.0 mg. of ac_2 , the resulting butter contained 0.86 mg. and the buttermilk (7.58 l.) contained 9.15 mg. Therefore 10.01 mg. of ac_2 was obtained in all, or 0.01 mg. more than was present in the cream. In another trial 10 kg. of cream (24.5% fat) produced 3.06 kg. of butter and 6.94 l. of buttermilk. The cream (10 l.) contained 24.2 mg. of ac_2 and the resulting butter contained none; the buttermilk (6.94 l.) contained 28.4 mg. or 4.2 mg. more than the original cream. This result was explained by a conversion of ame in the

cream to ac_2 by the carbonic acid present. To clarify this point 2 l. of sour cream containing 4.06 mg. of ac_2 was churned in a hand churn. The buttermilk was drawn completely and the resulting butter was washed three times with water. The buttermilk contained 6.14 mg. of ac_2 , the first wash water 3.16 mg. and the two succeeding wash waters none. The resulting butter contained 0.3 mg. ac_2 . In the trial, 5.54 mg. of ac_2 more than was present in the cream was accounted for. It appeared that ac_2 actually was formed during the churning process by oxidation of ame .

Results obtained by Alberti (1) indicated that considerable ame and ac_2 remained in the buttermilk.

Prill and Hammer (98) noted that certain general relationships applied to the distribution of ame and ac_2 between the butter and the buttermilk in all the churnings studied. The estimated mg. ac_2 in the buttermilk and the butter derived from 1 kg. of churning mixture agreed fairly well with the content of the partly churned mixture and the estimated mg. $ame + ac_2$ in the two products agreed fairly well with the content just before churning. The ac_2 contents of the butter were very nearly one-fifth those of the corresponding buttermilk. Since butter contains one-fifth serum, it would appear that ac_2 is contained entirely in the serum of butter. However, the $ame + ac_2$ content of the butter was, in most cases, only about one-tenth that of the buttermilk. The average of the estimated percentages of ac_2 in the churning mixtures that were retained in the butter was 8.8 per cent, and the corresponding value for $ame + ac_2$ was 4.5 per cent.

Partition of acetylmethylcarbinol and diacetyl between aqueous and fatty constituents of cream and butter. Orla-Jensen (85) stated that since the aroma of butter is produced outside the fat globules, it might be expected to wash out as easily as the lactic acid, but this is not the case. Isigny butter, which had a stronger aroma than any other make, was subjected to a very thorough washing during its manufacture. The explanation given was that butterfat can absorb essential oils and other odoriferous substances, both pleasant and unpleasant, and the aroma of the cream therefore passes into the fat globules. It was thought that the greater the proportion of fat globules present, the less aroma would be available for each globule and experience has shown that a high fat percentage is not conducive to production of an aromatic butter.

King (56) found a slow diffusion of ac_2 between the fat and serum phases of butter, ac_2 being dissolved in the fat fraction as well as in the serum. He believed that a slow diffusion of ac_2 from the serum to the fat occurred during storage.

Tapernaux (120) indicated that, during the course of cream fermentation, ac_2 is formed in the water and in the fat and divides itself between the serum and fat so that a part is eliminated in the buttermilk and also in the

wash water. Hammer (40, 41) reported that the ame and ac_2 in butter is mostly in the serum and only very little is in the fat.

According to Barnicoat (6) the final concentration of $\text{ame} + \text{ac}_2$ in butter is dependent on the amount of buttermilk retained. He believed that the compounds probably are held in the aqueous portion and are possibly absorbed on the protein rather than dissolved in the fat.

Michaelian and Hammer (73) found the amount of $\text{ame} + \text{ac}_2$ in the serum of butter was higher than the amount in the fat. Makar'in (67) stated that ac_2 in butter exists in the butter plasma which was considered to be made up of about one-half buttermilk. In one of eight trials, traces of ac_2 were found in the fat itself.

Davies (24, 25, 27) considered that since the vapor pressure of ac_2 is higher than that of ame a greater amount of ac_2 than of ame would be expected in the fat phase of cream. The amount of ac_2 in the fat, however, was less than the amount in the aqueous phase of butter. The author noted that a considerable amount of both ac_2 and ame occurs in the fat phase.

Mohr and Wellm (79) determined the amounts of ame and ac_2 in butter, butterfat and butter serum which was obtained by careful melting at 113°F . Butter containing 0.56 mg. ac_2 and 14.84 mg. $\text{ame} + \text{ac}_2$ per kg. had 0.52 mg. ac_2 and 5.14 mg. $\text{ame} + \text{ac}_2$ per kg. in the fat. Butter serum contained 1.24 mg. ac_2 and 61.1 mg. $\text{ame} + \text{ac}_2$ per l.

Virtanen (128) stated that ac_2 obviously is dissolved in the water portion of butter and is distributed between the buttermilk and the butter in the same proportions as water.

Results of Barnicoat (7) showed a higher ratio of ac_2 to ame in butter than in the cream from which it was churned. He considered that this observation might indicate a partition of the ac_2 with the fat but the data pointed to an actual development of ame in the butter. Neither ac_2 nor ame was concentrated in the fat during butter manufacture. Makar'in (68) stated that ame and ac_2 are not absorbed by the fat globules but are contained in the aqueous portion of butter. He believed that the aroma of butter was influenced by the concentration of aqueous constituents of the cream retained in the butter; as a rule this represented about 60 per cent of the total moisture content of the butter.

Hoecker and Hammer (47) studied the distribution of ac_2 and ame between "Wesson" oil and water or brine. In the mixtures the water or brine regularly contained higher concentrations of ac_2 and ame than the oil, the differences being greater with ame than with ac_2 . The percentage of ac_2 in a mixture that was contained in the oil was increased by sodium chloride in the water. The concentration of ac_2 in a mixture did not affect the percentage in the oil. Concentrations of ame in the oil were very low and the different concentrations in the mixtures gave essentially the same percentages in the oil. Studies made on the distribution of ac_2 and ame between

butterfat and water or brine showed that the water or brine regularly contained higher concentrations of the compounds than the fat; differences were greater with amc than with ac_2 . As the concentration of ac_2 in a mixture increased, the percentage in the fat did not change appreciably, while as the concentration of amc in a mixture increased, the percentage in the fat decreased. Studies on the distribution of ac_2 and amc between the fat and serum of unsalted and salted butter showed that the serum of butter contained higher concentrations of ac_2 and amc than the fat, with the greater differences again involving the amc. Averages of the percentages of ac_2 and amc contained in the fat were smaller with unsalted than with salted butter. The percentage of ac_2 contained in the fat was independent of the concentration in the butter, whereas the percentage of amc usually decreased somewhat as the concentration in the butter increased. Addition of a solution of ac_2 or a distillate of butter culture to salted butter resulted in essentially the same distribution of ac_2 as when the butter was made from cream containing butter culture. The amounts of ac_2 and amc in butter, as calculated from its composition and analyses of the fat and serum, agreed fairly closely with the determined amounts. In unsalted butter ac_2 and amc concentrations in the serum ranged from 0.25 to 3.55 ppm. and from 4.85 to 119.16 ppm., respectively, and those in the fat varied from 0.19 to 1.11 ppm. and from 0.88 to 6.86 ppm., respectively. The fat contained from 44.4 to 75.0 per cent of the ac_2 and from 26.2 to 46.8 per cent of the amc in the butter. In salted butter ac_2 and amc concentrations in the serum ranged from 0.07 to 2.00 ppm. and from 1.72 to 59.00 ppm., respectively, while those in the fat varied from 0.02 to 0.92 ppm. and from 0.29 to 4.87 ppm., respectively. Of the total ac_2 and amc in the salted butter, the fat contained from 48.5 to 78.4 per cent and from 23.6 to 46.0 per cent, respectively. The investigators concluded that comparative solubilities in the fat probably explain why with ac_2 the concentration in butter does not affect the percentage of the total that is retained in the fat, while with amc an increase in the concentration in butter decreases the percentage of the total that is retained in the fat. The low solubility of amc was believed to limit the amount taken up by the fat. Variations among samples of butter in the percentages of total ac_2 or amc that were contained in the fat were thought to be due to several factors, such as composition of the butter, physical state of the fat, churning procedure and degree to which water is dispersed in the butter; also, analytical errors involved in determining very small quantities of the compounds may be of minor significance. It also was concluded that since large percentages of the ac_2 and amc in cream at churning are removed with the buttermilk, higher concentrations of the compounds would be expected in the serum of butter than in the fat. Although concentrations in the fat were relatively low, the percentages of the compounds in butter that were contained in the fat were comparatively high because butter contains ap-

proximately 80 per cent fat. Partitioning of ac_2 and amc between the serum and fat was thought to reach an equilibrium in a relatively short time.

Influence of Reworking Butter

Reworking butter has an effect on the action of micro-organisms in it, including the butter culture types (64).

Barnicoat (7) stated that butter which develops off flavors during cold storage usually is considerably improved by reworking. This improvement has been thought to be due to formation of ac_2 ; results of the author, however, showed a slight decrease in ac_2 on reworking butter.

Influence of Storage of Butter

During the holding of butter there are various possibilities in connection with changes in the amc and ac_2 contents. The compounds may disappear through action of organisms or through chemical reactions. Also, under suitable conditions, they may be formed, particularly through action of the normal butter culture organisms; these organisms are much more active in unsalted than in salted butter (81).

Salted butter. Testoni and Ciusa (123) found that the amount of ac_2 in butter decreased on holding and that ac_2 did not develop in butter originally containing none. Tapernaux (120) stated that butter having an intense aroma tends to lose aroma as the holding period is extended and the aroma may even disappear. He suggested that the ac_2 is transformed into amc by reduction and then into 2,3-butylene glycol. Loss of aroma also was explained by the volatility of ac_2 . Davies (22) reported that ac_2 is lost more rapidly in butter of higher acidity.

According to Barnicoat (6) when butter was made from slightly ripened cream, with or without butter culture, the $amc + ac_2$ content remained fairly constant throughout the storage (193 days at 14° to 17° F.). Butter made from cream which had been slightly ripened with culture showed some conversion of amc to ac_2 ; however, in two samples less ac_2 was present at 6 months than at 3 months. All the butter made with culture contained considerable $amc + ac_2$ (0.7 to 2.3 ppm.). The ac_2 disappearing from butter during storage was considered lost through oxidation to simpler substances. Decomposition of ac_2 was greater in butter made without culture than in butter made with culture. In butter made with culture, a certain amount of ac_2 was lost and considerable of the original ac_2 was reduced to amc . The author believed that reduction of ac_2 to amc in butter made with culture was promoted by the reducing action of the culture organisms. The loss of ac_2 in butter made without culture, and to a less extent in butter made with culture, was considered due to oxidation by air. Decomposition of ac_2 in oxidized and tallowy lots of butter was found to be less than might be expected;

this indicated that the fat peroxides may not be as important in the degradation of ac_2 as is generally supposed.

Virtanen and Tarnanen (129) stated that when deterioration in butter is caused by organisms (for example, bacteria of the *Ps. fluorescens* group) ac_2 is lost very quickly; this is evident before other defects become noticeable.

In the studies of Slatter (115) $amc + ac_2$ usually was not produced in salted butter. No conspicuous increases occurred, even when very little salt was present. Salt appeared to retard the decrease in $amc + ac_2$ in butter held at various temperatures.

Davies (23) reported that butter made from ripened cream must stand 16 to 24 hours before the maximum aroma develops. This phenomenon occurred at any temperature between 30° and 60° F. Very little increase in aroma occurred at 15° F. The increase in ac_2 after the manufacture of butter was considered to be due to bacterial action on amc and to autooxidation of amc . In cream after ripening there is no oxygen in solution but, after churning and working the butter, conditions are different in that the water in butter is saturated with atmospheric oxygen. The organisms in the buttermilk droplets in butter were believed to readjust the $ac_2 : amc$ ratio to that existing in the early stages of cream ripening so that some of the excess amc is converted to ac_2 . The high acidity of the buttermilk droplets was thought to cause a slight organic acidity, due largely to oleic acid being split from the fat. Free oleic acid in the presence of atmospheric oxygen was thought to develop small amounts of peroxides which oxidize some of the amc to ac_2 . After 24 hours, the bacteria of the butter apparently utilize all the oxygen and there is no ac_2 formation. Davies concluded that the degree of flavor is lowered by a gradual oxidation of ac_2 to a flavorless product.

Barnicoat (7) observed no marked change in the ac_2 content of butter during frozen storage for 3 to 4 months. When butter was held at 40° F. for 7 to 10 days after manufacture, the ac_2 content decreased in some cases. Butter in which the greatest increases in ac_2 were observed were made from cream to which amc had been added and in which the culture was growing fairly vigorously at churning. Development of ac_2 in butter freshly made with culture was considered due to activity of the bacteria or their enzymes. The author concluded that the ac_2 content of butter made with culture, even after cold storage, generally is dependent on the concentration originally present in the cream. When ac_2 was added as the artificial substance, a decrease was observed; butter made with butter culture tended to increase slightly. Development of ac_2 in butter was not retarded, even when the butter was manufactured under reduced air pressure. An increase in ac_2 content of butter on holding 25 days at 40° F. was not considered due to atmospheric oxidation.

Bunger (16) found the ac_2 content of sour cream butter held at 62.6° to 71.6° F. remained practically the same during the first 4 days, whereas hold-

ing at 46.4° to 50° F. gave a slight increase in ac_2 . After 12 days at 46.4° to 50° F., or higher, a decrease was noted in all cases.° At 14° to 32° F. the ac_2 content was the same after 10 days.

According to Virtanen (128) the aroma of butter made from aerated cream, as well as that of butter made from non-aerated cream, greatly decreased during storage. Deterioration in butter made from aerated cream was no more extensive than in butter made from non-aerated cream; after 1 month both samples were graded approximately equal. Virtanen stated that disappearance of butter aroma during storage is an important problem for export countries, where butter cannot be sold fresh.

Matuszewski *et al.* (69) noted a distinct decrease and even disappearance of butter aroma in butter held 1 week at 50° to 59° F.; the butter decreased in both ac_2 and ame . Brioux and Jouis (13) reported that the amount of ac_2 in butter decreased rapidly. Within 15 to 18 days after manufacture, the ac_2 content of butter decreased to about one-tenth of its original value. One lot of butter containing 1.5 mg. ac_2 per kg. the day after churning contained only 0.05 mg. 26 days later.

Results of Davies (24, 25, 26) indicated that ripened cream butter requires from 12 to 24 hours before the full flavor develops. During this period ame is oxidized to ac_2 and an equilibrium is set up in the ratio of ac_2 to ame which is different from that in cream. Davies indicated that a different ratio is to be expected because there is practically no oxygen in solution in ripened cream while fresh butter is saturated with air.

Slatter and Hammer (117) did not find a significant increase in $ame + ac_2$ when salted butter was held at various temperatures. Butter containing 0.75 per cent salt showed a rather rapid disappearance of $ame + ac_2$ at 44°, 50° or 60° F. The amounts of $ame + ac_2$ in butter containing 1.0, 1.5 or 2.5 per cent salt remained fairly constant. Failure to obtain significant increases in the $ame + ac_2$ in salted butter was expected because of the restraining action of salt on the culture organisms.

Data obtained by Brioux and Jouis (14) indicated that in fresh butter ac_2 disappears rapidly when the butter is held. Butter containing 1.50 mg. ac_2 per kg. the day following manufacture, showed 0.60 mg. after 5 days, 0.13 mg. after 18 days and 0.05 mg. after 27 days. The ame content was 42.4 mg. per kg. the day following manufacture and 20.4 mg. after 27 days.

Pont (94) stated that full flavor does not develop in butter until approximately 24 hours after churning. There was no flavor development in butter held at the freezing point or lower.

Mohr *et al.* (78) found that sweet cream butter, with as much as 0.4 mg. ac_2 per kg. when fresh, contained practically none after 6 months in storage. When sour cream butter containing 1 to 2 mg. ac_2 per kg. was stored 6 months, the ac_2 content was reduced or remained constant. Various packing materials, such as parchment paper, transparent water-proof paper and

glass, had no effect on the ac_2 content. Storage of the butter in an atmosphere of carbon dioxide also had no effect. Iron and copper introduced into the butter from equipment increased the ac_2 content during storage but often caused off flavors.

Analyses on salted butter at various periods by Prill and Hammer (99) showed a rather high retention of ac_2 and amc , even at 70° F. Development of tallowiness at this temperature, which is common because of the great effect of relatively high temperatures on appearance of the defect, was not accompanied by a sharp decrease in ac_2 or amc contents.

Toth (125) noted that with butter prepared from acid cream the amount of aroma-producing substances increased for several days and then rapidly decreased. With butter made from sweet cream, a slow but constant decrease was observed; storage at low temperatures impeded these changes.

Hoecker and Hammer (47) obtained both increases and decreases in ac_2 with butter held 1 day at 40° F. and then 2 and 4 weeks at 0° or 35° F., the larger changes usually occurring at 35° F. Occasionally, increases in ac_2 contents after 2 weeks were followed by decreases after 4 weeks. Except in a few instances, the amc contents did not change appreciably.

Unsalted butter. Results obtained by Slatter (115) showed that $amc + ac_2$ commonly was produced in unsalted butter made with culture when the butter was held under favorable conditions, although the amounts in different lots held under the same conditions varied considerably. The largest production usually occurred at the highest holding temperature (60° F.) and in lots which developed the lowest pH. In several instances the amounts of $amc + ac_2$ in unsalted butter decreased during the first few days of holding and remained low, while in other cases a decrease was followed by an increase. A decrease regularly followed the maximum production of $amc + ac_2$. A larger production of $amc + ac_2$ occurred in the butter when 10, 15 or 20 per cent butter culture was added to the cream than when 5 per cent was used. In some cases addition of flavor-producing streptococci to the cream along with butter culture appeared to increase production of $amc + ac_2$ in butter during the holding.

Slatter and Hammer (117) found that when unsalted butter was held at 0°, 34° or 44° F. the amounts of $amc + ac_2$ sometimes increased and sometimes decreased, both increases and decreases being more definite at 44° F. than at lower temperatures. Increases commonly occurred at 50°, 60° or 70° F. At 50° and 60° F., maximum production usually was noted after 7 days. At 70° F. there was a decrease from the fourth to the seventh day. The pH of butter held at 44°, 50° and 60° F. was reduced gradually while at lower temperatures not much change occurred. A low pH in butter commonly was accompanied by a large production of $amc + ac_2$. Lowering of the pH and production of $amc + ac_2$ at relatively high temperatures sug-

gest that essentially the same changes take place in unsalted butter as in butter culture. The decrease in $\text{ame} + \text{ac}_2$ in unsalted butter is in agreement with the disappearance of these materials in butter cultures and is due, presumably, to reduction of ame or ac_2 to 2,3-butylene glycol by the flavor-producing streptococci. Development of $\text{ame} + \text{ac}_2$ at favorable temperatures suggests that a short ripening period may be desirable for commercial unsalted butter from the standpoint of obtaining flavor. However, these temperatures may also favor development of undesirable organisms.

Results of Mohr and Wellm (79) indicated that holding temperature exerts a decisive influence on the ac_2 and ame contents of butter. At 62.6° , 64.4° and 71.6° F. the amounts of ac_2 in sour cream butter remained practically constant for the first 4 days. Butter held at 50° F. usually showed an increase after 4 days. The ac_2 content decreased considerably in butter held 12 days above 46.4° F.; at 14° to 32° F. it remained practically constant, even after 12 days.

Prill and Hammer (99) found significant changes in ac_2 and in $\text{ame} + \text{ac}_2$ in unsalted butter held at 36° to 45° F. and at 70° F.; undoubtedly these were due to activity of the culture organisms. The changes involved both increases and decreases, as would be expected from the general relationship of the culture organisms to ame and ac_2 . An increase in the ac_2 content of unsalted butter was believed to be important in the flavor development which this product often undergoes; a subsequent decrease presumably is accompanied by a partial loss of flavor.

Analyses by Hedrick and Hammer (45) on lots of unsalted butter before and after holding showed the following: Of 56 lots of butter held 1 week at 36° to 40° F., 47 showed increases in ac_2 , 2 showed no change and 7 showed decreases; with the same lots, 38 showed increases in ame and 18 showed decreases. When the lots were held 3 days at 60° F. plus 4 days at 40° F., 45 showed increases in ac_2 and 11 showed decreases; with the same lots (minus 1), 37 showed increases in ame and 18 showed decreases. Of 43 lots of butter held 1 month at 36° to 40° F., 39 showed increases in ac_2 and 4 showed decreases; with the same lots (plus 1), 29 showed increases in ame , 1 showed no change and 14 showed decreases. The ac_2 and ame contents of butter held 3 days at 60° F. plus 4 days at 36° to 40° F. and of butter held 1 week at 36° to 40° F. were compared. The holding at 60° F. resulted in a higher ac_2 content in 42 of the 56 comparisons, no change in 1 and a decrease in 13; the ame content was higher in 40 of the 55 comparisons, the same in 4 and lower in 11.

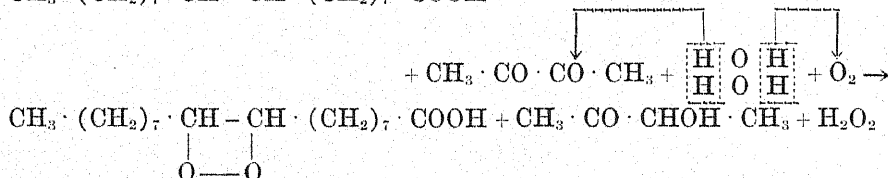
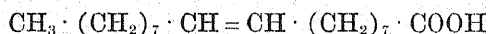
ACTION OF ACETYLMETHYLCARBINOL AND DIACETYL ON BUTTER

Recognition of the importance of ame and ac_2 from the standpoint of flavor and flavor development in butter at once suggested consideration of the relationship of these compounds to butter deterioration. Two angles

are involved, chemical and microbiological. Since ac_2 is rather highly oxidized, it readily acts on certain compounds that are easily oxidized and in this way might produce off flavors in butter. Also, ac_2 has germicidal properties and might influence growth of microorganisms in butter, thus preventing development of certain off flavors.

Chemical Considerations

King (56) studied the influence of ac_2 on butterfat by adding it to melted and filtered fat, obtained from sweet cream, in concentrations of 0.05, 0.01 and 0.005 per cent. The fat used was almost odorless and the ac_2 went into it on warming. One series of tests was conducted in diffuse daylight at approximately 64.4° to 73.4° F. while the other was carried out in the dark at 71.6° to 75.2° F. In the presence of air the butterfat became bleached and tallowy. The change was accelerated by light. Bleaching was first evident at the surface and progressed from there. In general, tallowiness appeared later than the bleaching. Rate of bleaching and degree of tallowiness were proportional to the amount of ac_2 present. King suggested that ac_2 may act on butterfat by oxidizing oleic acid to oleic acid peroxide, which then breaks up into various compounds having a pronounced tallowy odor and flavor, the ac_2 being reduced to amc or even to 2,3-butylene glycol. The following reactions were suggested as explaining the action of ac_2 on oleic acid:



The hydrogen peroxide reacts with hydrogen to form water. Fixation of oxygen on the colored material of the fat also was suggested since the carotinoid pigments are rich in double bonds.

According to Tapernaux (120) the more aroma butter possesses, the poorer is its keeping qualities. Kay (54) reported that oxidation of amc during storage of butter indicates oxidative changes in the butterfat. Butter with pronounced aroma had poorer keeping qualities than butter with less aroma. Davies (22) noted that one of the disadvantages of amc in butter is its action as an oxidation catalyst, accelerating deterioration of the fat.

Hammer (40) stated that ac_2 is highly oxidized and can react with various materials. Large quantities of it bleached butterfat and produced tallowy odors and flavors. Small amounts, such as are present in butter, were not considered a direct cause of these defects. Action of ac_2 was consider-

ably slower if an acid, such as lactic or sulfuric, was present. Hammer noted that ac_2 worked into butter may have a better opportunity to react with fat and other constituents than if it is largely present in the moisture droplets of the butter. Later, in commenting on King's studies (56) Hammer (41) pointed out that butterfat exposed to air develops a tallowy odor and flavor, the action being surprisingly rapid. He also suggested that observations on the action of ac_2 on butterfat cannot be directly applied to the action of ac_2 when it is added to butter for the purpose of improving the flavor because of the very small amounts used and probably for other reasons also.

An article published by the Polaks Frutal Works (93), and intended to refute a published statement (2), indicated that ac_2 does not exert a definite oxidizing action on fats but has a tendency to reduce.

Barnicoat (5) did not consider ac_2 in itself to be responsible for the poorer keeping qualities of ripened cream butter. Later, he (7) stated that ac_2 has no effect on the keeping qualities of butter made from "mild starter" cream. Added ac_2 (6 ppm.) was not important in promoting deterioration of butter held 3.5 months at 14° F. Barnicoat noted that marked deterioration of high acid butter is related to low pH and not to its high ac_2 content.

Results obtained by Davies (23) led to the conclusion that with low-flavored butter it is unlikely the traces of ac_2 (0.1 to 0.3 ppm.) present will initiate autoxidation in the absence of considerable serum acidity; nor is this the case with medium-flavored butter (0.4 to 0.8 ppm. ac_2) or full-flavored butter (0.9 to 2.0 ppm. ac_2). Much larger concentrations of ac_2 (6.0 ppm.) were very active in promoting fat autoxidation, the compound being more active in this respect than an equivalent amount of oleic acid. Davies also reported (24) that tallowiness in full-flavored butter is not due so much to ac_2 as to conditions associated with manufacture of such butter. Later (25, 26, 27), he stated that full-flavored butter does not keep well but develops fishiness and oxidative rancidity much more rapidly than butter made from sweet or neutralized cream. The aroma constituent was not considered responsible for these defects but rather the high acid content. The amount of ac_2 in butter was regarded as too small to initiate or catalyze oxidative changes.

Pont (94) indicated that the peroxides formed as a result of the liberation of oleic acid oxidize ac_2 to flavorless compounds during butter deterioration. Further changes involve breakdown of lecithin, resulting in fishy flavor, and oxidation of butterfat, resulting in stale and tallowy flavors.

The peroxide numbers on samples of butterfat which were heated to 219.2° F. and held there for 8 hours were determined by Ritter and Nussbaumer (101). Addition of 0.1 or 1 per cent ac_2 to the butterfat resulted in a significant increase in peroxide number; the addition decreased the oxidation resistance of the butterfat. When butterfat contained 0.1 or 1

per cent added ac_2 , it was much more susceptible to oxidation than control samples or samples containing less than 0.01 per cent ac_2 . The greater the susceptibility of untreated butterfat to oxidation, the greater the accelerating influence of ac_2 .

Wiley (136) stated that the presence of butter culture organisms in butter favored oxidation of the fat at cold storage temperatures. This was thought to be due to some product of bacterial metabolism acting as a pro-oxidant; the product was not ame or ac_2 .

In referring to the work of King (56) Prill and Hammer (99) noted that he used pure butterfat with a relatively high concentration of added ac_2 and, in some cases, rather drastic treatments, such as exposure to light, while in butter it is probable that much of the ac_2 is in the water phase rather than entirely in the fat. In general, it appeared that the amount of ac_2 ordinarily encountered in butter has no significant effect on promotion of chemical defects and that other factors, such as copper content of the butter and pH of the serum, are of more importance in this connection.

Barnicoat (7) compared the vitamin A and carotene contents of lots of butter (made from portions of the same batch of cream) after 22 months at 14° to 17° F.; ac_2 was added to two of the lots during working at the rate of 4 ppm., or more than 12 times the concentration usually found in "mild starter" butter. The loss of vitamin A and its precursor carotene in both sweet cream and "mild starter" butter containing unusually large proportions of ac_2 was not greater than in control butter churned from sweet cream.

Hunziker and Cordes (48; p. 357) studied several thousand churnings of butter made from unripened cream in which ac_2 (approximately 3 to 4 ppm.) was added to the butter. After holding the butter 2 to 9 months, there was no indication of bleaching, tallowy flavors or other flavors such as might be attributable to oxidation by ac_2 .

Toth (125) stated that too high a concentration of ac_2 is disadvantageous in the storing of butter since, on its reduction to ame , fats become rancid.

Microbiological Considerations

Lemoigne (61) stated that ac_2 in relatively small and variable amounts can retard development of micro-organisms. Experiments of Lévy-Bruhl and Cado (62) showed that *Staphylococcus aureus* was killed in bouillon by 0.1 per cent ac_2 but not by 0.04 per cent. Various organisms, including streptococci, pneumococci, gonococci and typhoid, paratyphoid, diphtheria and coliform types, were killed by 0.04 per cent but not by 0.02 per cent.

AMOUNTS OF ACETYLMETHYLCARBINOL AND DIACETYL IN VARIOUS DAIRY PRODUCTS

Over the years in which there has been an active interest in ame and ac_2 from the standpoint of flavor development in butter and related prod-

ucts, the methods of determination have changed greatly. For the detection of relatively small amounts of these compounds, colorimetric methods now are commonly used, instead of the less sensitive gravimetric methods (43; p. 156). This change in analytical procedure should be recognized in any consideration of the reported ame and ac_2 contents of dairy products.

Milk

Testoni and Ciusa (123, 124) did not find ac_2 , ame or 2,3-butylene glycol in fresh milk; Mohler and Herzfeld (76) and also Pien *et al.* (91) did not detect ac_2 in it. Schmalfuss and Werner (112) reported that milk intended for household use contained no detectable amounts of ac_2 or ame , that is, less than 0.000024 per cent.

Ac_2 was not found in spontaneously soured milk by Testoni and Ciusa (123); later studies (124) indicated that old milk may contain some 2,3-butylene glycol. Schmalfuss and Barthmeyer (111) detected both ac_2 and ame in sour milk, about ten times more ame than ac_2 being present. Pien *et al.* (91) did not find ac_2 in acid coagulated milk.

Skimmilk

When 1 l. was used for the distillation, Krenn (58) did not find ac_2 in skimmilk. Schmalfuss and Werner (112) reported neither ac_2 nor ame in fresh skimmilk. Mohr and Wellm (79) believed that skimmilk contains both ac_2 and ame , although only to a very small extent (0.17 mg. ac_2 per l.).

Cream

Testoni and Ciusa (123) detected ac_2 in centrifuged cream. According to Michaelian and Hammer (73) sweet cream frequently contains a small amount of $\text{ame} + \text{ac}_2$; however, cream freshly skimmed from milk low in bacteria showed none in 200 g. Pien *et al.* (91) found no ac_2 in fresh cream. Slatter (116) stated that since a high acidity is necessary for production of ac_2 , it is not found in sweet cream. Krenn (58) did not detect ac_2 in sweet cream when 1 l. was used for the distillation.

Tapernaux (120) reported that ac_2 can be detected in ripened cream, especially when the ripening is not preceded by pasteurization. According to Virtanen and Tarnanen (129) ripened cream contains practically no ac_2 but considerable amounts of ame . Pien *et al.* (91) obtained variable results when testing spontaneously soured cream for ac_2 ; the samples always showed some ac_2 (about 1.5 mg. per l.). Cream several weeks old showed about 1.3 mg. per l. Schmalfuss and Werner (112) found the ac_2 content of sour cream to be 0.000024 per cent. Slatter (116) stated that only small amounts of ac_2 are found in sour cream.

Additional data on the ame and ac_2 contents of cream intended for butter are given in the consideration of the influence of cream acidity on the ame and ac_2 contents of butter.

Other Products

Pien *et al.* (91) did not detect ac_2 in aqueous solutions of lactic acid from fermented milk. Considerable quantities of lactic acid gave a slight test for ac_2 and the distillate from 25 ml. syrupy lactic acid gave a positive reaction corresponding to 0.3 mg.

Schmalfuss and Werner (112) obtained a positive test for ac_2 on the material recovered by dry distillation of milk sugar. Dry distillation of citric acid did not give a positive test. The authors (112) found the ac_2 content of commercial margarine to be 0.00014 per cent.

Butter

van Niel *et al.* (126) found from 0.0002 to 0.0004 per cent ac_2 in fine butter. Schmalfuss and Barthmeyer (110) analyzed four samples of butter representing different conditions of feeding the producing animals and obtained the following weights of nickel salt (43; p. 156) equivalent to ac_2 per kg.: 0.0006, 0.0002, 0.0003 and 0.0001 g. The quantity of ac_2 in the butter appeared to be correlated with the intensity of the aroma. Testoni and Ciusa (122) stated that melted and pasteurized butter, as well as margarine, do not contain ac_2 , whereas fresh butter contains about 0.00051 per cent. They believed the presence of ac_2 was a measure of the freshness of the butter. Later (123), they did not find ac_2 in properly prepared butter.

According to Tapernaux (120) fresh unpasteurized butter contains from 0 to 50 g. ac_2 in 100 kg.; pasteurized butter contained traces. Using a method capable of detecting 0.02 or more g. ac_2 per kg., Vizern and Guillot (130) obtained negative tests on butter. Hammer (40) found more amc than ac_2 in butter made with butter culture. Davies (22) stated that butter made from ripened cream may contain 0.05 to 0.5 ppm. of ac_2 . Mohler and Herzfeld (76) reported that butter of normal aroma contains 0.0002 to 0.0004 per cent ac_2 .

Michaelian and Hammer (73) examined 56 lots of butter for $amc + ac_2$. With a gravimetric method, 4 of them showed none while the remaining 52 yielded from 0.1 to 3.45 mg. nickel salt per 200 g. Steuart (118) found that the quantity of ac_2 in butter ranged from 0 to 5 ppm., depending on the presence and proportion of aroma bacteria in the souring cream. Testoni and Ciusa (124) stated that Italian butters do not normally contain ac_2 ; its presence was regarded as accidental. Pien *et al.* (91) found very little or no ac_2 in good butter (trace to 1 mg. per kg.).

Barnicoat (7) reported no correlation between the ac_2 content of butter and the score. Butter made with culture, which was quite acceptable to the graders, generally contained 0.2 to 0.4 ppm. ac_2 and 1 to 3 ppm. amc . "Mild starter" butter contained about eight times the ac_2 in sweet cream butter, but only one-fourth of that in highly flavored butter.

Of the butter samples analyzed by Brioux and Jouis (13), 76.3 per cent contained ac_2 in amounts ranging from a trace to 0.5 mg. per kg.; the re-

mainder contained from 0.51 to 1.5 mg., except in one instance for which the value was 2.5 mg. The amc contents were much higher, usually from 10 to 30 mg. per kg., with a maximum of 69 mg.

Davies (24) reported that the absence of ac_2 gives a flavorless butter, 0.2 to 0.6 ppm. gives a mild flavor and 0.7 to 1.5 ppm. gives a full flavor; 2 ppm. ac_2 gives a strong flavor and aroma which is harsh and repulsive to butter graders. Makar'in (67) stated that the aroma of butter is pronounced with an ac_2 content of 0.00048 per cent in the plasma. An ac_2 concentration as low as 0.0013 per cent produced an odd, sharp odor.

According to Matuszewski *et al.* (69) butter made from cream that had been inoculated with a culture of *S. diacetylactis* contained about 5 mg. nickel salt per kg.

Mohr and Wellm (79) noted that the method of producing butter, whether from sweet or sour cream, is of major importance with regard to the ac_2 content. Sweet cream butter contained considerably less ac_2 than sour cream butter. As much as 0.2 mg. ac_2 per kg. and 0.36 mg. amc + ac_2 was found in sweet cream butter. In various lots of German first-class butter, the ac_2 content varied from 0.34 to 1.66 mg. per kg. and the amc + ac_2 content from 3.73 to 20 mg.

Of the 130 samples of butter investigated by Pien *et al.* (92), 11 per cent contained ac_2 in amounts ranging from 0.5 to 1.0 mg. per kg., 47 per cent contained from 0.1 to 0.5 mg. and 42 per cent contained less than 0.1 mg.

Schmalfuss and Werner (112) found the ac_2 content of German butter made in August to be 0.00003 per cent. Slatter and Hammer (117) reported that the amount of amc + ac_2 in butter at the time of churning varied with the amount of butter culture used. Dehove and Dessirier (29) stated that all butter naturally contains a small amount of amc.

Brioux and Jouis (14) examined samples of fresh butter and found that they normally contained a small quantity of ac_2 , usually from 0.05 to 0.5 mg. per kg.; in rare cases as much as 2.5 mg. was found. Butter made in cooperative or industrial creameries contained considerably more ac_2 than butter made on farms. The amc contents were considerably higher than the ac_2 contents; usually butter contained between 10 and 30 mg. per kg. but some lots contained as much as 69 mg. Amc did not appear to give rise to an appreciable quantity of ac_2 .

Alberti (1) found the amount of ac_2 in butter varied from a trace to 0.4 mg. per kg. when the samples were from the same source. The amc contents were from 10 to 40 times the ac_2 contents.

According to Mohr *et al.* (78) fresh sweet cream butter contained up to 0.4 mg. ac_2 per kg. and sour cream butter from 1 to 2 mg. Salted and unsalted butter showed no difference in ac_2 contents.

In comparisons involving addition of equal percentages of regular and aerated or modified aerated (p. 88; p. 89) culture to pasteurized sweet cream,

Prill and Hammer (98) noted that butter made with the latter types of culture invariably had the higher ac_2 contents (0.38 to 1.0 mg. per kg. compared to 0.14 to 0.38 mg.). However, the higher values were much lower than they would have been if the ac_2 contents of the butter were proportional to those of the cultures.

Toth (125) found that Hungarian butter contained 0.05 to 1.5 mg. per cent ac_2 and 1.5 to 4.2 mg. per cent amc .

Hoecker and Hammer (47) noted that butter made without butter culture or with *S. aromaticus* contained only small amounts of ac_2 and amc , whereas butter made with butter culture, *S. citrovorus*, *S. paracitrovorus*, *S. diacetylactis*, *S. citrophilus* or an unidentified organism commonly contained appreciable amounts.

BUTTER CULTURE DISTILLATE AS A SOURCE OF FLAVOR FOR BUTTER

Amc in various materials is readily converted to ac_2 by oxidation with some such reagent as ferric chloride, and the ac_2 thus formed, as well as that originally present, can be distilled out. Distillates obtained from butter culture with this general procedure (often called starter distillates) have been suggested for producing flavor in butter, the distillates being worked into the butter during the normal working process. Because a butter culture contains much more amc than ac_2 , a distillate contains much more ac_2 than the volume of culture from which it was obtained. However, some of the amc distills over before its conversion to ac_2 (72) and appears as such in the distillate.

Since volatile acids, and possibly other volatile compounds, contribute to the flavor of butter culture, a culture distillate may contain materials other than ac_2 that are desirable from the standpoint of butter flavor. On the basis of the present knowledge, volatile acids appear to be of most importance in this connection.

Ruehe and Ramsey (107) noted that the flavor constituents of butter cultures, including ac_2 and amc , are removed by steam distillation. Addition of the distillate to sweet cream before churning yielded butter of intensified aroma. Similar results were obtained by adding the distillate directly to butter. Butter cultures used for steam distillation were grown in such a manner that they contained increased amounts of the flavoring constituents.

Use of an ac_2 concentrate was suggested by Davies (23). The concentrate was prepared by distillation of ac_2 from butter culture and addition of the distillate to neutralized butter culture. Such preparations contained 300 times more ac_2 than ripened cream.

According to Ruehe (105) the ac_2 content of butter culture distillate does not change appreciably, even after 63 days at 40° F. When butter was made with various amounts of distillate, most judges preferred that containing less than 1 part ac_2 in 200,000 parts of butter.

The scheme suggested by Ruehe (106) for controlling flavor in butter consists of selection of cultures which actively produce lactic acid and ferment citrates when grown in milk, adoption of methods which are conducive to high yields of ac_2 , steam distillation of the culture, standardization of the ac_2 content of the distillate and addition of the distillate to the churned butter at the time of salting. Advantages of the suggested method are that the distillate can be prepared in the laboratory and supplied to various plants, flavor intensity can be adjusted to demands of the trade, high-flavored butter with good keeping qualities can be produced, butter has a low bacterial count and culture distillate is more economical than butter culture. In a series of comparisons involving use of 2.5 per cent butter culture and various amounts of ac_2 as butter culture distillate, the butter containing ac_2 as distillate in the ratio of 1 part ac_2 to 400,000 parts of butter scored highest when fresh; after 2 months at 40° F., the butter containing ac_2 as distillate in the ratio of 1 part ac_2 to 800,000 parts of butter scored highest. Similar results were obtained when the lots of butter were held at -10° F. for 8 months and scored at intervals of 2 months.

DETECTION OF ADDED DIACETYL IN BUTTER

In the examination of butter, detection of ac_2 added as such, or in the form of a flavor concentrate, sometimes is attempted. It is complicated by the fact that ac_2 commonly is present in butter through use of butter culture and/or cream containing ac_2 .

Davies (22) noted that a relatively high ac_2 content and a low acidity in butter should indicate that ac_2 had been added artificially. Later, he (27) reported that analytical evidence for detection of added synthetic ac_2 in butter is unsatisfactory. The two main evidences of such addition are acidity of the butter serum and ratio of ac_2 to amc . The author stated that butter serum having a pH of 6.4 to 6.8 should not contain a detectable amount of ac_2 . The ratio of ac_2 to amc in normal butter was considered to be from 1:15 to 1:20. Butter culture distillates had ratios of 5:1 to 2:1.

The ratio of ac_2 to amc in butter was suggested by Barnicoat (5) as of value in the detection of added ac_2 . Bacteriological examination was proposed as a guide for determining whether butter culture had been used in the cream from which the butter was churned.

Pien *et al.* (92) stated that since none of the butter examined contained more than 1.0 mg. ac_2 per kg., samples containing larger amounts had ac_2 added to them artificially.

Hoecker and Hammer (47) studied pure milk cultures of various streptococci, including *S. citrovorus*, *S. paracitrovorus*, *S. diacetylactis*, *S. citrophilus*, an unidentified organism and *S. aromaticus*. With each species the ratio of ac_2 to amc varied in the different trials; frequently the ac_2 was much higher in proportion to the amc than with butter cultures. The results

suggest the dangers in using the ac_2 to amc ratio in detecting added ac_2 in butter.

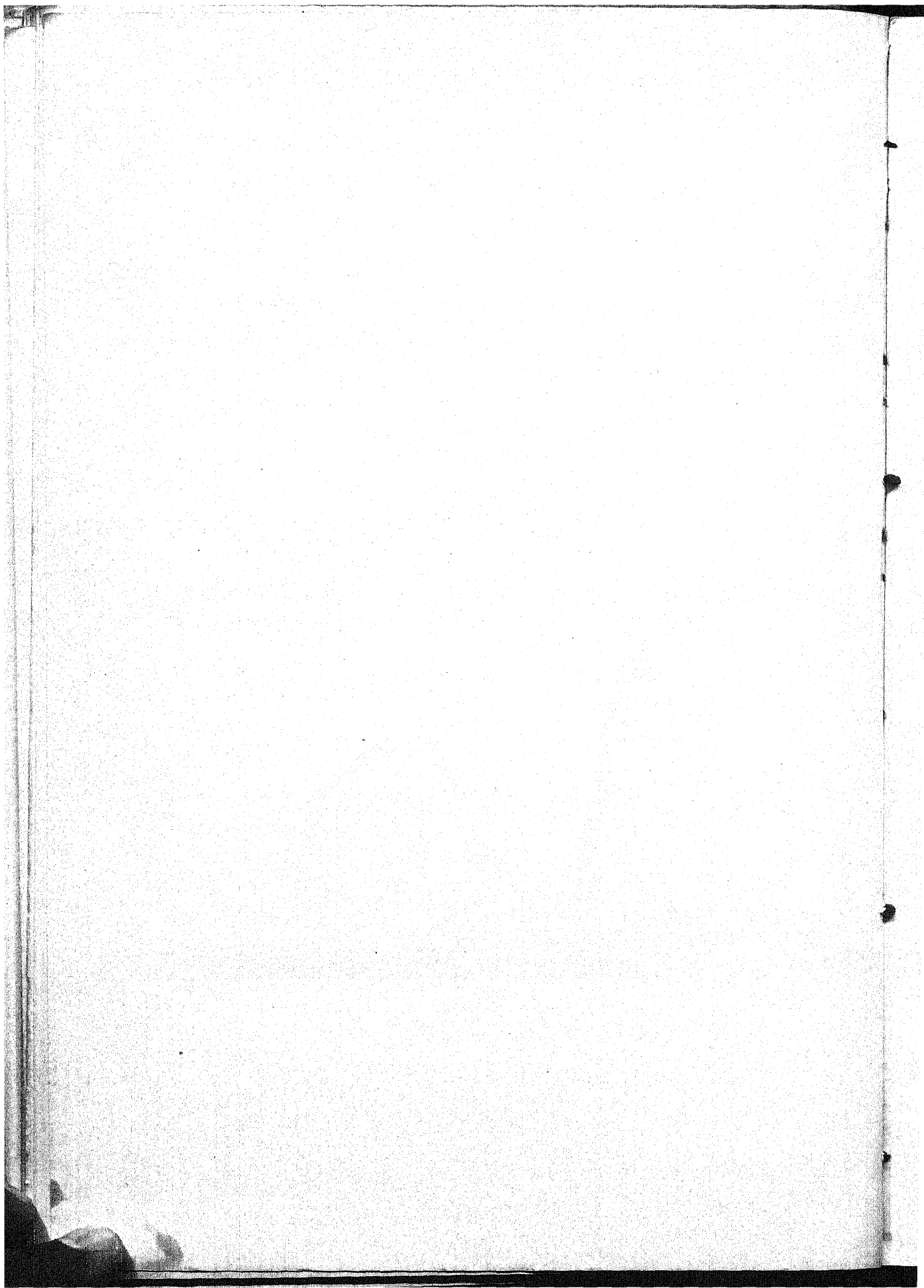
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A SATISFACTORY METHOD OF SHIPPING DAIRY BULL SEMEN LONG DISTANCES*

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The methods of collecting, storing, and handling bull semen after collection have been widely investigated in recent years for the purpose of extending the use of valuable sires through artificial insemination (1, 3, 5, 6). Satisfactory progress has been made in this field especially in its application to large cooperative associations where the bulls are within easy reach of the cows to be inseminated. It is often desirable, however, to breed certain cows to bulls which are a great distance away. Shipment by the ordinary means of transportation available may require two days or more in many instances. This situation presents a different transportation and storage problem than is encountered in shorter shipping distances.

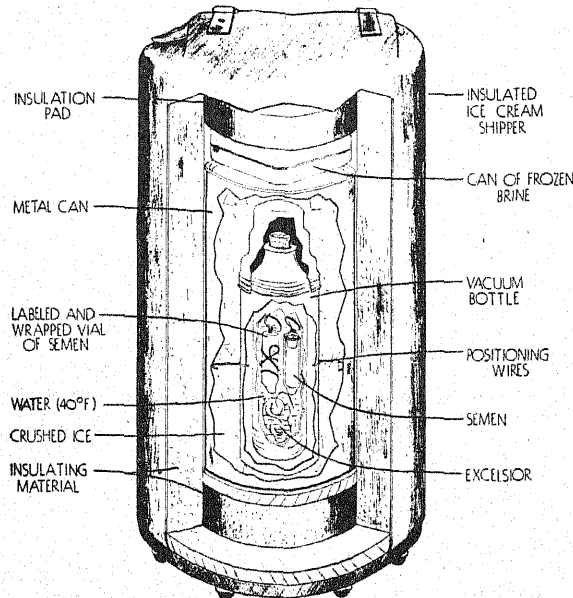
Numerous investigators (1, 2, 3, 5, 6) have shown that the proper temperature for storage of bull semen is below 45° F. and preferably at or slightly below 40° F. or 5° C. Hence, some reliable practical method of refrigeration and temperature control is necessary. Glass vacuum bottles alone may be used, but in addition to being very fragile, they are unsuited for lengthy shipment because of the relatively short period at which a temperature as low as 40° F. may be maintained. A light cardboard shipping container, described by Salisbury (4) has proved suitable in artificial breeding association work where the shipping time does not exceed 24 hours. Ice-filled thermal jugs likewise have been used successfully for short-time shipment. In addition to providing only a short safe shipping time, these types of packages have given difficulty because the semen is in close contact with ice which in cold weather may cause the temperature of the semen to drop too low. An investigation was therefore conducted to find a shipping method that could be depended upon to maintain proper refrigeration for 48 hours or more and at the same time not harm the semen by too low temperatures. A prime consideration was to adopt a container which would withstand the rigors of shipping. The type of package finally adopted and preliminary results from its use are reported below.

The requirements of long distance shipping were; 1) adequate refrigeration of the package, 2) protection of the semen from the intense cold of the refrigerant, and 3) insulation of the refrigerant against the external temperature. It was desired to meet these requirements as simply as possible so that the method would be quickly and easily available to all who wished

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to use it. For this reason utilization was made of existing equipment entirely. The refrigerant used was mainly crushed ice with a can of frozen brine used for extra hot weather. A small vacuum bottle filled with water at 40° F. was used to contain the vials of semen; and commercially available small insulated shipping packages such as are commonly used for ice cream were used as external insulation. These were combined as diagrammed in figure 1.



A PACKED SEMEN SHIPPER

FIG. 1. A cut-away diagram of the packed semen shipper.

The cooled or partially cooled semen in an insulated, rubber-wrapped vial was placed in a vacuum bottle of water at 40° F. A few strands of excelsior in the bottle prevent undue bobbing of the vials during transit. The vacuum bottle, with supporting wires to position it at the center, was put into a metal can and chipped ice was packed around it as shown in figure 1. The can was sealed and placed in a small ice cream shipper. Best results were secured when the can fit in the shipper tightly or was packed in with insulating material. If extra refrigeration is desired a can of frozen brine may be placed on top of the can. The shipper should be packed quickly, closed, and shipped. The packed shipper weighs between 15 and 20 pounds, depending upon how much ice is used; so it can be easily shipped by express or parcel post.

This shipper has maintained a satisfactory semen storage temperature for more than 84 hours at summer temperatures (80° F.) and several ship-

ments (two in hot August weather) have been successfully made in which the package was three days in transit. Maximum holding time can be obtained by precooling the shipper and using a maximum of ice plus the frozen brine pad.

The semen is protected from the severe cold of the ice by means of the water in the vacuum bottle. The size of the vacuum bottle and the amount of ice used can be varied for different outside temperature conditions. In summer shipments a one-half pint vacuum bottle provided enough water insulation so that the temperature of the semen did not fall below 34° F. Also, a large amount of ice could be packed around this small bottle. In cold winter weather, however, only a small amount of ice was needed in the can to insure proper refrigeration. A full can of ice around a one-half pint bottle was not satisfactory with the outside temperature below 32° F. because the temperature of the semen dropped to 32° F. Three different shipments of semen known to be fertile under proper storage conditions produced no pregnancies when they had been cooled to 32° F. By using a pint bottle in place of the one-half pint bottle and packing the can only half full with crushed ice, the temperature of the water did not fall below 34° F. and the fertility of the shipped semen was maintained very satisfactorily. Therefore, the recommended procedure is to use a pint vacuum bottle and a small amount of ice in winter conditions and to use a one-half pint bottle with a maximum amount of ice in hot summer conditions.

The glass vacuum bottles in these containers are subject to shattering under very rough handling. With ordinary care and handling, however, no breakage has occurred. If breakage of the bottle does occur, the chances are still good for the semen to come through in satisfactory condition because of the refrigerant being outside of the bottle.

Twenty-four shipments of semen have been made by this method in the course of twelve months' investigations. When the proper precautions as outlined above have been observed, the shipments have been successful in delivering semen with motility as good or better than that obtained from the same semen stored at 40° F. in a refrigerator. Furthermore, the few inseminations made have yielded satisfactory results. Eight conceptions have been secured from twelve inseminations using semen ranging from 48 to 130 hours of age regardless of its motility rating. The semen used ranged from 70 to 90 per cent motility when packed and was from University of Missouri bulls known to have a low insemination-per-conception ratio. The inseminations made with the semen which was cooled to 32° F. in transit were not included in this summary. Six inseminations which were made with semen which had been stored 144 hours or more (all of very poor motility) were infertile. Results have been, therefore, such as would be expected from the use of properly handled stored semen. The success in shipping semen by the method described will depend largely upon the ability of the semen to maintain fertility during storage at 35 to 45° F.

SUMMARY

A simple, dependable, and practical method of shipping semen long distances is described. The package consists of a small insulated ice cream shipper, a water-tight metal can to fit the shipper, crushed ice, and a vacuum bottle. Satisfactory semen storage temperatures (35 to 50° F.) were maintained in this shipper for 84 hours at atmospheric temperature of 80° F. Results of inseminations with shipped semen requiring 48 to 72 hours in transit were very satisfactory for stored semen.

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THE ACCURACY OF THE MOJONNIER METHOD FOR ESTIMATING MILK FAT IN MILK AND CREAM

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Laboratory technicians have inquired about the degree of accuracy that can be achieved in estimating milk fat in milk by the Mojonnier method. They have also questioned the relative accuracy of single or duplicate determinations on individual samples. Data are presented for milk and cream to answer these questions.

LITERATURE REVIEW

The literature related to this study is the result of comparing the accuracy of the various volumetric methods with the Røese-Gottlieb method and its mechanized modification, the Mojonnier method, for estimating milk fat in milk and cream. The literature is cited if the data are complete and sufficient to make a contribution of statistical value.

Phillips (4) estimated 50 samples of unpreserved milk in duplicate for milk fat. The individuals in 29 duplicates agreed, 15 differed by 0.01 per cent, 5 by 0.02 per cent and 1 by 0.03 per cent milk fat. Dahlberg, Holm and Troy (1) made 55 estimations for milk fat in duplicate. Some of the estimations were made on replicate samples in different laboratories by the Røese-Gottlieb and the Mojonnier methods. Of the 55 estimations, 14 agreed, 8 differed by 0.01, 18 by 0.02, 9 by 0.03, 1 by 0.04, 2 by 0.05, 1 by 0.07, 1 by 0.22 and 1 by 0.29 per cent milk fat. Fisher and Walts (2) made 32 estimations and approximately 81 per cent of the individuals in duplicates did not differ by more than 0.04 per cent milk fat.

METHODS

The methods of preparing the samples and estimating them for milk fat have been described (3). After preparing the samples of milk and cream, they were transferred directly to the extraction flasks and weighed (3). Four technicians participated in this study.

It was necessary to obtain some information on the accuracy of estimating milk fat in replicate samples. One hundred estimations were made on 100 samples of the same milk that was produced during the month of June. These individual samples were prepared by agitating 40 quarts of fresh, raw milk at 8-10° C. for about two minutes. As the milk was being

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decrease in milk fat when the samples are held for longer periods, but an observation will be mentioned. Samples 81-88, inclusive, were prepared and weighed into the extraction flasks at the end of the second day. Due to the late hour, the flasks containing the milk were allowed to remain in the laboratory over night and the ether extractions were completed the next day. No sour odors could be detected from these 8 samples the next morning. These samples (81-88) averaged 3.68 per cent milk fat, which is significantly lower than the average for the 100 samples. The other 17 samples in the same group averaged 3.71 per cent milk fat.

The frequency distribution in table 2 shows that 82 of the estimations vary from the mean by 0.02 per cent or less and 18 by more than 0.02 per cent milk fat. Therefore, the odds are 82 to 18 or 4.56 to 1 that individual

TABLE 2

Frequency distribution of estimations for milk fat on 100 replicate samples of unpreserved milk

Per cent milk fat	Frequency
3.66	2
3.67	2
3.68	5
3.69	3
3.70	6
3.71	19
3.72	24
3.73	23
3.74	10
3.75	4
3.76	2
	<hr/> 100

Mean = 3.72 per cent.

Standard deviation = 0.02 per cent.

estimations will not vary from the mean by more than about 0.02 per cent and will agree within 0.04 per cent milk fat. Furthermore, 76 per cent of the estimations are in the frequency groups of 3.71 to 3.74, a difference of 0.03 per cent milk fat.

The results in table 2 should serve as a basis to predict the accuracy that may be attained in routine analyses. To test the validity of this hypothesis, the differences between individuals for each of 341 duplicate estimations on fresh milk were calculated. These estimations were made at regular intervals over a period of about two years. The results are given in the third column of table 3. It is evident that nearly 77 per cent of the individuals in duplicates agreed within 0.03 per cent milk fat and approximately 86 per cent of them within 0.04 per cent milk fat; this agrees closely with the results in table 2.

To show the comparative accuracy of single and of duplicate estimations, it is assumed that the average of duplicates will give the most accurate

results. The variation of the individuals from their mean is one-half of the difference between single determinations for each sample of milk. These calculations were made on the same 341 samples of milk and the results are given in the fourth column of table 3. Eighty-six per cent of the individuals in duplicates did not vary from their means by more than 0.02 per cent milk fat, about 94 per cent by not more than 0.03 per cent milk fat and about 98 per cent by not more than 0.04 per cent milk fat.

Bimonthly composite milk samples preserved with bichloride of mercury were estimated for milk fat in duplicate. These samples were obtained from two milk plants during the summer months. Sixty samples were obtained

TABLE 3

Frequency distribution of differences between the individuals in duplicates and between individuals and the means of their duplicates for milk fat estimations on 341 samples of unpreserved milk

Frequency	Per cent	Differences between individuals	
		Duplicates	Means of duplicates
		Per cent milk fat	
70	20.53	0.00	0.000
94	27.57	0.01	0.005
63	18.48	0.02	0.010
36	10.56	0.03	0.015
32	9.38	0.04	0.020
16	4.69	0.05	0.025
12	3.52	0.06	0.030
6	1.76	0.07	0.035
6	1.76	0.08	0.040
2	0.59	0.09	0.045
1	0.29	0.10	0.050
1	0.29	0.11	0.055
1	0.29	0.12	0.060
1	0.29	0.13	0.065
341	100.00		

from Plant A. These samples were in excellent physical condition because they had been properly refrigerated and cared for in the milk plant during the 15-day period. Another set of sixty samples was obtained from Plant B. These samples were not in good physical condition. Some of them showed varying degrees of fat destabilization and mold growth. The results in table 4 show that 95 per cent of the individuals in duplicate estimations from Plant A agreed within 0.03 per cent milk fat and all of them within 0.05 per cent; furthermore, the differences between individual estimations and the means of their duplicates on all the samples are less than 0.03 per cent milk fat. As might be expected, the variability of the results was greater from Plant B, about 73 per cent of the individuals agreeing within 0.03 per cent milk fat and 85 per cent within 0.04 per cent. The increased variability of the results from Plant B can be attributed to the difficulty of obtaining a

TABLE 4

Frequency distribution of differences between individuals in duplicate estimations for milk fat on 120 samples of preserved milk

Differences milk fat per cent	Plant			
	A		B	
	Frequency	Per cent	Frequency	Per cent
0.00	13	21.67	6	10.00
0.01	19	31.67	19	31.67
0.02	19	31.67	8	13.33
0.03	6	10.00	11	18.33
0.04	7	11.67
0.05	3	5.00	2	3.33
0.06	2	3.33
0.07
0.08	3	5.00
0.09	2	3.33
	60	100.01	60	99.99

representative sample, because of the destabilized condition of the fat emulsion. The technician who made the estimations on the preserved samples was able to achieve greater accuracy than any of the other three who participated in this work.

Two hundred and forty-two samples of unpreserved cream containing 20-45 per cent of milk fat were estimated for milk fat in duplicate at regular intervals over a period of about two years. It is evident from table 5 that approximately 86 per cent of the individuals in duplicate estimations agreed within about 0.30 per cent milk fat. Approximately 86 per cent of the individuals in the duplicates did not vary more than 0.15 per cent milk fat and about 97 per cent not more than 0.30 per cent from their means.

TABLE 5

Frequency distribution of differences between the individuals in duplicates and between individuals and the means of their duplicates for milk fat estimations on 242 samples of unpreserved cream

Frequency	Per cent	Differences between individuals	
		Duplicates	Means of duplicates
		Per cent milk fat	
103	42.56	0.0-0.09	0.0 -0.045
68	28.10	0.1-0.19	0.05-0.095
38	15.70	0.2-0.29	0.10-0.145
8	3.31	0.3-0.39	0.15-0.195
12	4.96	0.4-0.49	0.20-0.245
7	2.89	0.5-0.59	0.25-0.295
3	1.24	0.6-0.69	0.30-0.345
1	0.41	0.7-0.79	0.35-0.395
1	0.41	0.8-0.89	0.40-0.445
1	0.41	0.9-0.99	0.45-0.495
242	99.99		

DISCUSSION

An accuracy can be achieved with single estimations on normal milk samples that may not vary more than 0.03 per cent milk fat in at least 75 per cent of the cases and 0.04 per cent milk fat in at least 82 per cent of the cases (tables 2, 3). An accuracy can be achieved with duplicate estimations that may not vary more than 0.02 per cent milk fat in about 86 per cent of the cases, 0.03 per cent milk fat in about 94 per cent of the cases, and 0.04 per cent milk fat in about 98 per cent of the cases (tables 3, 4).

For cream, approximately 86 per cent of the individuals in duplicate estimations did not vary more than 0.29 per cent milk fat, and about 86 per cent of the individuals in duplicates did not vary more than 0.145 per cent milk fat from their means.

The comparative accuracy of the Mojonnier method on milk and cream can be calculated. Assuming that cream contains 40 per cent milk fat, then the variation between individuals in 86 per cent of the duplicate estimations would be 0.725 per cent of the total milk fat ($0.29 \div 0.40 \times 100 = 0.725$). Using this same method of calculating and assuming that milk contains 4 per cent of milk fat, then the variation between individuals in at least 82 per cent of the cases would be one per cent of the total milk fat ($0.04 \div 4 \times 100 = 1$). Therefore, a slightly higher degree of accuracy can be achieved on cream than on milk.

The condition of the fat emulsion affects the degree of accuracy that can be achieved. Approximately 73 per cent of the individuals in duplicates agreed to 0.03 per cent milk fat from Plant B (table 4) as compared to 95 per cent of the individuals from Plant A. The fat emulsion in the samples from Plant B showed varying degrees of destabilization. Duplicate estimations should be made on milk samples if the fat emulsion is abnormal. For commercial and routine analyses, single estimations on individual samples of normal milk may give sufficient accuracy for practical purposes; however, when a high degree of accuracy is desired, duplicate estimations should be made. The importance of the results to be achieved should determine whether single or duplicate estimations for milk fat are to be made on samples of milk and cream.

CONCLUSIONS

1. Single estimations of the milk fat content of milk by the Mojonnier method can be expected to give an accuracy within 0.03 and 0.04 per cent milk fat in at least 75 and 82 per cent, respectively, of the cases.

2. The mean of duplicate estimations can be expected to give an accuracy within 0.02 and 0.03 per cent milk fat in approximately 86 and 94 per cent, respectively, of the cases for normal milk.

3. Single estimations of the milk fat content of normal cream by the Mojonnier method can be expected to give an accuracy of about 0.30 per cent milk fat in about 86 per cent of the cases.

4. Duplicate estimations for normal cream can be expected to give an accuracy within 0.30 per cent milk fat in about 97 per cent of the cases.

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THE MILK AND BUTTERFAT PRODUCTION RESPONSES TO SHARK LIVER OIL IN THE RATION*

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Reports on the results of feeding shark liver oil to dairy cattle are conflicting.

Deuel and co-workers (1, 4) reported that the daily feeding of 30 to 60 cc. of shark liver oil increased the milk and butterfat production of Guernsey cows approximately 10 per cent. This amount of shark liver oil furnished from 700,000 to 1,400,000 I.U. of Vitamin A.

Rupel, Boyer and Phillips (8) found that the daily feeding of 25 cc. of shark liver oil with a Vitamin A potency of 7,500 I.U. and 15,000 I.U. per gram had no effect on the milk production and fat percentage of Holstein and Guernsey cows. These authors reported that the daily feeding of 90 cc. of a 15,000 I.U. shark liver oil caused a noticeable drop in the amount of 4 per cent fat-corrected milk.

More recently Jensen *et al.* (5) reported that daily feeding of shark liver oil to furnish from 117,500 to 1,350,000 I.U. of Vitamin A resulted in no increase in milk or butterfat production of Jersey, Guernsey, Holstein, and Brown Swiss cows. It was found that the daily feeding of as much as 90 cc. of shark liver oil tended to increase the normal rate of decline in milk production with the advance of lactation.

In view of these conflicting reports the results presented in this paper are offered as additional information.

EXPERIMENTAL

Twelve cows, 6 Jerseys and 6 Holsteins, were used in the experiment. These cows were divided into 4 groups; group I, Holstein supplement, group II Jersey supplement, group III, Holstein control and group IV, Jersey control. The groups were so divided that the production of each was approximately that of its control group.

All groups were fed the same basal ration, consisting of alfalfa hay, grain mixture and sunflower silage, which was replaced by sweet clover silage after 8 weeks. The grain mixture consisted of barley, oats, wheat bran, linseed oil meal, cottonseed meal, dried molasses beet pulp, iodized salt, and steamed bone meal.

All groups were fed the basal ration during a preliminary period of 4 weeks. Beginning with the fifth week the rations of groups I and II were

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supplemented with 60 cc. daily of shark liver oil with a Vitamin A potency of 25,000 I.U. per gram.

The duration of the experiment was 16 weeks, beginning on January 3, 1942, and ending April 30, 1942.

The shark liver oil supplement was discontinued April 18, 1942, but the cows were kept under observation until April 30, 1942.

Daily milk weight records were kept on all cows during the preliminary and the oil feeding periods. Composite milk samples were taken daily from each cow and the per cent butterfat determined by the Babcock method. Once a week an additional composite milk sample was taken from each cow for Vitamin A and carotene determination.

The butterfat was extracted from the milk and treated prior to digestion by the method of Olson *et al.* (7). Vitamin A and carotene determinations on the unsaponified residue were made according to the method of Koehn and Sherman (6).

RESULTS

The daily feeding of 60 cc. of shark liver oil with a Vitamin A potency of 25,000 I.U. per gram had no effect on milk or butterfat production. These data are presented in figure 1 and table 1.

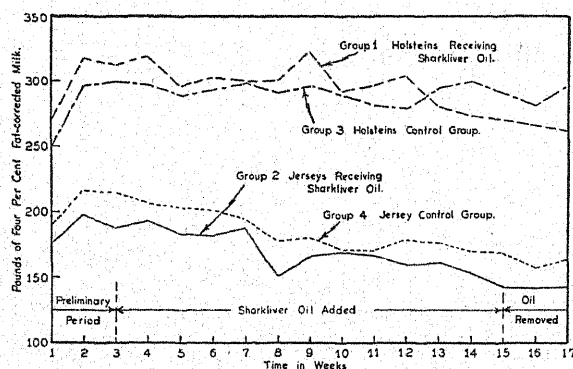


Figure 1. Average pounds of four per cent fat-corrected milk produced per week.

Fig. 1. Average pounds of four per cent fat-corrected milk produced per week.

The data in table 1 show that the feeding of shark liver oil caused a marked increase in the Vitamin A content of the butterfat. This increase was approximately 280 per cent for the Holstein cows and 305 per cent for the Jersey cows. The vitamin A content of the butterfat reached its greatest value the second week after the cows received the supplement. After this the Vitamin A content of the butterfat dropped somewhat, but was maintained at a considerably higher level than that of the cows receiving no supplement.

The carotene content of the butterfat of the cows receiving the supplement gradually decreased during the course of the experiment (table 1).

TABLE 1

The effect of shark liver oil feeding on the milk and fat production and the vitamin A and carotene content of the milk fat of Holstein and Jersey cows

Period week ending	Group I Holstein Basal ration + 60 cc. shark liver oil			Group III Holstein Basal ration		
	Av. weekly milk yield, lbs. 4% F.C.M.	Av. vita- min A, I.U./gm. milk fat	Av. caro- tene, micro- grams/gm. milk fat	Av. weekly milk yield, lbs. 4% F.C.M.	Av. vita- min A, I.U./gm. milk fat	Av. caro- tene, micro- grams/gm. milk fat
Preliminary period						
1- 9-42	269.7	248.1
1-16-42	318.7	24.2	4.4	297.0	33.2	3.9
1-23-42	312.6	18.6	3.6	299.8	16.9	2.6
1-30-42	319.6	20.3	3.0	293.6	19.8	2.4
Experimental period						
2- 6-42	297.6	52.6	3.2	289.0	20.5	2.0
2-13-42	303.2	69.0	3.6	293.3	23.7	2.9
2-20-42	301.5	44.3	3.0	299.5	22.0	2.9
2-27-42	301.4	57.7	2.9	292.4	20.7	2.8
3- 6-42	291.6	54.4	2.9	297.8	20.5	2.8
3-13-42	293.9	66.5	2.2	290.2	21.3	2.4
3-20-42	287.7	64.9	3.2	282.0	20.1	2.7
3-27-42	304.7	53.0	2.4	279.3	20.3	2.9
4- 3-42	280.7	57.9	2.4	294.6	20.9	2.5
4-10-42	274.0	45.4	1.8	299.8	16.3	3.4
4-17-42	271.4	52.8	1.9	296.3	15.7	2.4
4-24-42*	266.2	55.0	2.7	280.5	21.2	3.6
5- 1-42	262.3	42.1	3.6	297.2	31.0	4.3
	Group II Jersey			Group IV Jersey		
Preliminary period						
1- 9-42	175.5	190.0
1-16-42	198.3	18.7	6.2	216.7	20.5	6.0
1-23-42	187.5	15.3	5.3	214.0	16.6	4.1
1-30-42	193.1	12.4	4.2	206.3	12.6	3.3
Experimental period						
2- 6-42	184.2	43.7	4.5	204.1	24.2	3.9
2-13-42	181.7	71.5	4.5	202.9	29.3	4.3
2-20-42	225.1	39.2	3.9	195.7	18.6	4.2
2-27-42	181.3	48.1	3.9	177.9	23.5	4.5
3- 6-42	166.2	44.3	3.2	180.7	23.8	3.9
3-13-42	168.8	53.4	3.0	171.2	24.7	3.6
3-20-42	166.1	49.3	3.6	170.9	24.9	3.7
3-27-42	159.2	39.4	2.8	178.1	28.9	3.8
4- 3-42	161.8	53.6	3.5	176.9	25.2	4.3
4-10-42	153.2	39.8	2.9	170.2	22.8	4.3
4-17-42	142.9	42.3	3.1	165.9	22.9	3.8
4-24-42*	141.8	51.4	2.9	190.6	24.7	4.9
5- 1-42	142.1	29.8	4.6	164.8	25.9	6.1

* Shark liver oil removed from ration.

There was an average reduction of 22 per cent in carotene content of the Holstein butterfat, and 33 per cent in the carotene content of the Jersey butterfat.

DISCUSSION

The data presented show that the daily administration of 60 cc. of shark liver oil furnishing a daily intake of 1,350,000 I.U. of Vitamin A had no effect on milk or butterfat production. This supports the results of Jensen *et al.* (5) and is contrary to the findings of Deuel and co-workers (1, 4).

The increase in the Vitamin A content of the milk is in agreement with the results of Deuel and co-workers (2, 4) and Jensen *et al.* (5).

While the increase in Vitamin A content of milk fat was not as marked as that reported by Deuel, it was significant. The decrease in carotene content of the butterfat supports the results of Deuel (3) and of Jensen *et al.* (5).

SUMMARY

1. The daily feeding of 60 cc. of shark liver oil furnishing 1,350,000 I.U. of Vitamin A had no effect on milk or butterfat production of either Holstein-Friesian or Jersey cows.

2. The daily feeding of 60 cc. of shark liver oil resulted in a 280 to 305 per cent increase in the Vitamin A content of the milk fat.

3. The feeding of shark liver oil caused a 22 per cent average decrease in the carotene content of the Holstein milk fat, and a 33 per cent average decrease in the carotene content of the Jersey milk.

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A 2×2 FACTORIAL DESIGN FOR DOUBLE REVERSAL FEEDING EXPERIMENTS

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Investigators in the dairy field have for years made liberal use of the double-reversal design for conducting feeding trials. This design employs the procedure of using two groups of cows (A and B) and starting period I with the A group on ration X and the B group on ration Y. At the end of each period the rations are reversed; thus, in period III the groups are fed the same rations as in period I. In the analysis the production during period II is compared to the average for periods I and III. The comparison thus made is considered appropriate because of the usual decline in production of cows as the lactation progresses. The assumption is made that if other factors remain under control the cows will produce as much in period II as the average of periods I and III.

One of the strongest criticisms against employing the double reversal design is that there is a residual, or carry-over effect, caused by the previous rations fed. In 1941 Cochran, Autrey and Cannon (1), in a discussion of a double change-over design presented a method for correcting this influence. A partial correction for this carry-over effect is also made possible by discarding the records for a preliminary period at the beginning of each feeding period.

A method for determining the significance of the differences found in the reversal or switch-back trials was presented by Brandt in 1938 (2). An extension of this test is herein presented so as to permit the experimenter to compare two sets of factors at the same time—such as two concentrates and two roughages—with an appropriate method of testing the significance of the differences found between each set of factors. The design where applicable can employ the same number of cows to answer two questions as is commonly used to answer a single question. Caution should be exercised in its use, however. The experimenter should know from previous knowledge or experience that the differences between the two roughages is likely to be of about the same magnitude while on each of the two concentrate mixtures.

THE EXPERIMENTAL PLAN

The particular design presented grew out of an attempt to use a limited number of cows in two feeding trials to compare two kinds of hay and two concentrate mixtures. Rather than conduct separate trials it was decided

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¹ The author is greatly indebted to Dr. W. G. Cochran of the Statistical Laboratory of Iowa State College for guidance in developing the analysis as herein presented.

to combine the trials with the major group, A or B, getting the same kind of hay (alyce or lespedeza)² and the subgroups, a and b, differing in the kind of concentrate, either three parts corn and one part cottonseed meal or three parts dehydrated sweet potato meal and one part cottonseed meal. Twenty

TABLE 1

*Alyce clover vs. lespedeza and corn vs. dehydrated sweet potato meal in the dairy ration
(Production of 4% equivalent milk)*

Hay group	Grain group	Cow No.	Period I	Period II	Period III	$P_1 - 2P_2 + P_3$
			P_1	P_2	P_3	
A			Alyce + s. pot.	Lesp. + corn	Alyce + s. pot.	
	a	1	369.7	337.6	303.9	- 1.6
		2	376.1	380.8	413.8	+ 28.3
		3	440.0	376.0	407.4	+ 95.4
		4	375.0	362.2	344.5	- 4.9
		5	248.5	257.5	278.5	+ 12.0
		Sums	1809.3	1714.1	1748.1	+ 129.2
			Alyce + corn	Lesp. + s. pot.	Alyce + corn	
	b	1	206.4	196.1	210.6	+ 24.8
		2	486.0	423.4	469.7	+ 108.9
		3	425.0	350.7	373.6	+ 97.2
		4	342.5	302.9	362.9	+ 99.6
		5	474.9	422.5	442.3	+ 72.2
		Sums	1934.8	1695.6	1859.1	+ 402.7
B			Lesp. + s. pot.	Alyce + corn	Lesp. + s. pot.	
	a	1	182.1	207.7	199.7	- 33.6
		2	436.1	501.0	430.5	- 135.4
		3	341.3	378.0	338.18	- 76.6
		4	373.9	427.8	374.3	- 107.4
		5	383.1	404.6	345.8	- 80.3
		Sums	1716.5	1919.1	1688.4	- 433.3
			Lesp. + corn	Alyce + s. pot.	Lesp. + corn	
	b	1	257.1	253.3	239.5	- 10.0
		2	436.7	461.3	487.9	+ 2.0
		3	489.3	484.3	441.0	- 38.3
		4	308.4	332.3	307.8	- 48.4
		5	235.5	245.0	248.6	- 5.9
		Sums	1727.0	1776.2	1724.8	- 100.6

cows were used in the trial with 10 in each hay group and these were subdivided into two five-cow concentrate groups. Table 1 presents the plan

² The lespedeza hay used was approximately one grade lower in quality than was the alyce clover hay. The comparisons made are for the purposes of presenting a method of analysis rather than to give experimental results.

along with the resulting milk yields for the cows for each of the three periods. Each period consisted of 21 days; 7 days as a preliminary period and 14 days as a test period.

THE ANALYSIS

1. *An Example Showing Significant Differences*

The present experiment conforms to a factorial pattern, an experimental design described in detail by Yates (4). This particular factorial design involves variations in two sets of factors, so is designated as a 2×2 factorial, and has the interaction between the two sets of factors confounded with cows. Our notation on the factors involved is:

<i>First factor</i>	<i>Second factor</i>
1 Alyce clover	1 Sweet potato
2 Lespedeza	2 Corn

Using this notation the sub-group totals in table 1 are:

$$\begin{aligned} A(a) \ 11 - 22 &= +129.2 \\ A(b) \ 12 - 21 &= +402.7 \\ B(a) \ 21 - 12 &= -433.3 \\ B(b) \ 22 - 11 &= -100.6 \end{aligned}$$

The sub-total differences are derived by adding sums for Periods I and III and subtracting twice the sum for Period II. Thus, 11 - 22, or the sub-group difference in production between alyce + sweet potatoes and lespedeza + corn equals $1809.3 - 2(1714.1) + 1748.1$ or +129.2. A study of the subgroup totals immediately gives evidence that alyce clover + corn was the best combination, for this ration as shown in B(a) excelled lespedeza and sweet potatoes by 433.3 pounds of milk. This type of comparison, however, involves possible first-order interactions which are confounded with cows in this experiment. These interactions are ignored and the first problem at hand is to determine the differences between the two kinds of hay and the two concentrate mixtures. They are:

$$\text{alyce} - \text{lespedeza} = 129.2 + 402.7 - (-433.3) - (-100.6) \text{ or } 1065.8$$

$$\text{S. pot.} - \text{corn} = 129.2 + (-433.3) - 402.7 - (-100.6) \text{ or } -606.2$$

These differences show in one case the superiority of alyce clover over lespedeza and in the other the inferiority of sweet potato meal as compared to corn. The next procedure is to determine the significance of these differences.

The Analysis of Variance employing the calculation of the sum of squares for a single degree of freedom as described by Snedecor (3), will first be used. In terms of a single difference ($P_1 - 2P_2 + P_3$) the sum of squares for these components are:

$$\frac{(1065.8)^2}{20^*} = 56,796$$

$$\frac{(-606.2)^2}{20} = 18,373$$

* The denominator equals nk or the number of major groups (2) \times the number of cows in each group (10) or 20. See (3).

The individual cow differences as well as the sub-group totals under the heading $P_1 - 2P_2 + P_3$ in table 1 are employed in computing the error sum of squares, thus: $(-1.6)^2 + (28.3)^2 + (95.4)^2 \dots + (5.9)^2 - 1/5 [(129.2)^2 + (402.7)^2 + (-433.3)^2 + (-100.6)^2] = 26,703$ with 16 degrees of freedom. This gives for the analysis of variance:

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Alyce vs. lespedeza	1	56,796	56,796	34**
S. pot. vs. corn	1	18,373	18,373	11**
Error	16†	26,703	1,669

The analysis shows that both sets of factors were significantly different at the one per cent level**; with alyce clover excelling lespedeza, and corn excelling sweet potato meal.

An analysis involving the use of group means and testing the significance by the use of the standard error will next be presented. Table 2 shows a 2×2 table with totals. The treatment means per cow per period with differences, and the respective standard errors of these differences are also given.

TABLE 2
Milk yields by treatments—with means and differences

	Alyce	Lespedeza	Mean	Difference
S. potatoes	355.5	339.8	347.6	15.2 ± 4.567
Corn	381.6	344.0	362.8	
Mean	368.6	341.9		
Difference	26.7 ± 4.567			

Calculating these means on a per cow per period basis involves using sub-group totals from table 1; thus, for alyce + s. pot. = $1809.3 + (2 \times 1776.2) + 1748.1/20$ or 355.5. In like manner the other three totals in table 2 are calculated. From this the variance of a figure in this 2×2 table becomes:

$$5/400 [1^2 + 2^2 + 1^2] \times (\text{variance per cow per period}) =$$

$$3/40 c^2, \text{ where } c^2 = \frac{1,669\dagger}{6}. \text{ This gives } 20.86.$$

The standard error of the difference = $\sqrt{20.86}$ or 4.567

$$t = \frac{\text{Mean difference}}{\text{S.E.}} = \frac{26.7}{4.567} = 5.85^{**}$$

Likewise

$$\frac{15.2}{4.567} = 3.33^{**}$$

The values of t , as was the case with F in the analysis of variance, are significant at the one per cent point. The t and F tests are, of course, identical.

† The error term has 16 degrees of freedom, four from each group.

‡ The method for obtaining 1669 is given for Error Variance in the analysis of variance and 6 is the sum of exponents $1^2 + 2^2 + 1^2$.

2. An Example Showing non-Significant Differences

Liveweight changes as well as milk and butterfat yields are used as "measuring sticks" in double reversal feeding trials. Their use in the experiment just described furnishes data to illustrate a case where the differences observed failed to be significant at the five per cent level. The use of the analysis of variance to test this significance will be presented.

Table 3 presents the liveweight changes for each cow and the sums for the sub-groups. The effects of the rations are shown by

$$\text{Alyce-lespedeza} = (-64 - 434) - (+33) - (-118) \text{ or } -413$$

$$\text{S. pot.-corn} = (-64 + 33) - (-434) - (-118) \text{ or } 521$$

TABLE 3
Summary of liveweight changes

Hay group	Grain group	Cow No.	Period I	Period II	Period III	$P_1 - 2P_2 + P_3$
			P_1	P_2	P_3	
A			Alyce + s. pot.	Lesp. + corn	Alyce + s. pot.	
	a	1	+38	+18	-25	-23
		2	+10	-15	+42	+82
		3	-3	+45	-10	-103
		4	+9	+10	-12	-23
		5	-14	+8	+33	+3
		Sums	+40	+66	+28	-64
			Alyce + corn	Lesp. + s. pot.	Alyce + corn	
	b	1	-7	+29	-5	-70
		2	-16	+12	+4	-36
		3	-64	+79	-15	-237
		4	+22	+13	-1	-5
		5	+1	+37	-13	-86
		Sums	-64	+170	-30	-434
B			Lesp. + s. pot.	Alyce + corn	Lesp. + s. pot.	
	a	1	+13	-29	+9	+80
		2	+20	+2	+5	+21
		3	-25	+23	+0	-71
		4	+12	-9	+22	+52
		5	+13	+28	-6	-49
		Sums	+33	+15	+30	+33
			Lesp. + corn	Alyce + s. pot.	Lesp. + corn	
	b	1	-1	+14	-18	-47
		2	+24	+9	-10	-4
		3	+27	+35	+19	-24
		4	+4	+3	+22	+20
		5	+9	+38	+4	-63
		Sums	+63	+99	+17	-118

It appears from these differences that lespedeza excelled alyce hay and that sweet potato meal excelled corn in maintaining liveweight.

In terms of single differences ($P_1 - 2P_2 + P_3$) the sum of squares for the two sets of factors becomes:

$$\frac{(-413)^2}{20} = 8,528$$

$$\frac{(521)^2}{20} = 13,572$$

The error sum of squares is $(-23)^2 + (82)^2 \dots (-63)^2 - 1/5 (-64)^2 + (-434)^2 + (33)^2 + (-118)^2 = 70,850$

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Alyce vs. lesp.	1	8,528	8,528	1.9
S. pot. vs. corn	1	13,572	13,572	3.1
Error	16	70,850	4,428

In this test neither sets of factors are significantly different at the five per cent level. The size of the F value for sweet potato meal vs. corn might lead the experimenter to be suspicious that a real difference did exist and cause him to repeat the experiment in an effort to determine the accuracy of the first information secured.

SUMMARY AND CONCLUSIONS

An efficient method of using the double reversal experimental design to answer two questions with a limited number of cows is presented. The design makes it possible to compare two roughages and also two concentrate mixtures in the same feeding trial. The appropriate use of this design is where previous knowledge or experience indicates that the first degree interaction is unimportant, *i.e.*, that differences between the concentrate mixtures will be approximately the same while on each roughage.

Appropriate tests for measuring the differences based on a 2×2 factorial design are presented. These tests utilize the analysis of variance or the t-test with identical results. Examples given include milk yields showing significant differences between both roughages and concentrates, and live-weight changes, with non-significant differences between each set of factors.

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ANNOUNCEMENT

THIRTY-NINTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION COLUMBUS, OHIO, JUNE 20-22, 1944

NOTE REGARDING THE PROGRAM

In planning the program for the Columbus meeting of the American Dairy Science Association, the officers and members of the Program Committee are endeavoring to focus the program upon the problems of America at War and the problems following, during the peace to come. In order to formulate a program of the most usefulness and interest, it is essential that the committee receive the titles of proposed papers at as early a date as possible, but certainly not later than April 1. Titles will be carefully considered by the committee and abstracts of the papers, which, unless otherwise stated, are assumed to be not longer than 12 minutes, must be in the hands of the Chairman, Professor H. P. Davis, Dairy Husbandry Department, University of Nebraska, Lincoln 1, Nebraska, not later than June 1.

TENTATIVE PROGRAM OUTLINE

TUESDAY, JUNE 20, 1944

Morning Session

Welcome by representative of the host institution.
Response and address by President Dahlberg.
Address by guest speaker.

Afternoon Session

Sectional meetings.
4:00 P.M. Section business meetings.

WEDNESDAY, JUNE 21, 1944

Morning Session

Section meetings.
11:00 A.M. Section business meetings.

Afternoon Session

General session on post war problems.

THURSDAY, JUNE 22, 1944

Morning Session

Joint symposium—Production and Extension Sections.
Manufacturing symposium.

Afternoon Session

General session on Latin American Dairying.

3:30 P.M. Association business meeting.

Evening Session

Annual Banquet.

H. P. DAVIS

Chairman, Program Committee

NEW PROCEDURE TO NOMINATE CANDIDATES FOR OFFICE

In accordance with the provisions of the revised constitution adopted last year, your president has appointed a committee on nominations whose names appeared in the January issue of the Journal and are again listed below. It is the duty of this committee of which J. H. Frandsen is chairman to send its report to the secretary not later than April 1. The secretary will then mail out the ballots to secure a final vote before the annual meeting. The results of the election will be announced at the annual meeting.

As a member of the Association, it is your obligation to express your wishes for officers and directors to any member of this committee. Two candidates for vice-president and four for directors will be nominated. The present vice-president automatically becomes president. The committee on nominations will give consideration to the wishes of the members as expressed by correspondence and will also consider additional candidates. They have been advised by your president to study the list of past officers and directors (see pages 803-804 of the August Journal) to plan to secure good geographic distribution and to recognize the desirability of representation from all lines of activity of our members. This information has been given in detail to the committee for their guidance.

It was the intention of our Association to make the election of officers just as democratic as possible, and this can be done only if members do promptly give their opinions freely to the committee on nominations.

A. C. DAHLBERG, *President*

COMMITTEE ON NOMINATIONS

J. H. FRANDSEN, *Chairman*

H. A. RUEHE

J. F. KENDRICK

C. N. SHEPARDSON

H. P. DAVIS

JOURNAL OF DAIRY SCIENCE

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NUMBER 3

RELATIONSHIP OF FAT ACIDITY TO RANCIDITY IN HOMOGENIZED RAW MILK*

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The action of milk lipase on butter fat is generally believed to produce at least two detectable changes: first, the production of free fatty acids, and second, the production of rancid or rancid-like flavors. The production of the rancid flavor is attributed to free butyric acid and other lower fatty acids such as capric, caprylic, and caproic. Furthermore, the appearance of the rancid flavor is expected whenever milk fat undergoes lipolysis, indicating that the glycerides of the lower fatty acids are always attacked by lipase under normal conditions. Some efforts have been made to correlate the extent of fat splitting with rancid flavor development (1, 5, 7), but information on this relationship is by no means complete nor has it been applied to conditions in which the lipase activity is accelerated by homogenization.

The amount of lower fatty acids liberated from butterfat by lipase action may be expected to be relatively small inasmuch as the glycerides of the lower fatty acids constitute a comparatively small portion of the total fat and, also, since the work of Willstatter and Memmen (10) indicates pancreatic lipase to have slight affinity for the lower esters.

Although the quantity of the lower fatty acids involved in lipase activity is small, the effect of these acids on flavor may be marked. Grossfeld and Battay (3) observed butyric acid could be detected by smell when present in a ratio of 1:12,500. In the case of dairy products, the actual type of flavor produced by lipase activity may not always be well defined and may vary with conditions. Relatively slight lipase activity may result in flavor defects which are not typically rancid (4, 7).

Results which have been reported showing the relationship between the acid degree of the fat (ml. of N NaOH per 100 grams of fat) and the rancid flavor in the original product from which the fat was obtained are not in total agreement. Fouts (1) in his study of commercial butter, could find no direct relationship between rancidity of the butter and the acid degree of the butterfat. The acid degrees of fat from non-rancid butter ranged

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from 2.9 to 11.6, whereas values for rancid butter ranged from 2.4 to 14.0. In those samples which were rancid, there was no relationship between intensity of rancidity and free fatty acid content. Recently, Krukovsky and Herrington (6) and Jack, Tarassuk, and Scaramella (5) conducted controlled experiments in which butter was manufactured from milk having naturally active lipase. Krukovsky and Herrington found the rancid flavor to appear in butter between acid degrees of 0.75 and 2.0 with the lower value being near the flavor threshold of rancidity. Jack *et al.* concluded rancidity of butter was recognized organoleptically when the acid degree approached a value of 2.0 and that direct correlation exists between acid degree and rancid flavor.

In experiments with milk, Krukovsky and Herrington (7) determined "the acid degree at which rancidity could first be recognized as such." Although some variations occurred, the threshold at which rancidity was recognized in the milk was approximately at an acid degree of 0.8. However, the data indicated a tendency for the judges to lower the flavor score of milk as a result of lipase action even before the nature of the defect was recognized. Samples with an acid degree above 1.4 were graded in the low-fair and poor class.

The studies of Krukovsky and Herrington (7) were concerned with normal milk and do not necessarily apply to homogenized milk in which lipolysis is accelerated. This is indicated by the results of Gould and Trout (2) which showed homogenization to result immediately in greatly increased acid degrees in the fat, and also, by the observations of Trout and Scheid (9) that certain commercial plants homogenized raw milk and then pasteurized it immediately without any detectable flavor change. Trout and Scheid also demonstrated the feasibility of this practice. Lane and Hammer (8) observed the flavor of cheese made from a mixture of raw skimmilk and raw homogenized cream to be good even though the fat acidity was high (acid degree 9.7 in one case, 8.2 in another). However, flavor production in cheese is not necessarily analogous to that of milk.

The experiments herein reported were conducted with the view of securing information on the relationship between the acidity of fat from homogenized raw milk and the rancid flavor. The problem was investigated from two angles: (a) by homogenizing raw milk and then making fat and flavor examinations at different intervals, (b) by homogenizing fat possessing different acid degrees and secured from non-homogenized and homogenized raw milk into pasteurized skim milk and then examining the mixture for the rancid flavor.

EXPERIMENTAL PROCEDURE

Milk used in these studies was selected from milk delivered to the College Creamery, care being used to obtain milk practically free of off-flavors. Homogenization of this milk was at 700 pounds pressure and at 37° C. Fat

was secured for analysis by separation, churning and filtering. Ten gram samples were titrated for free fatty acids with 0.05 N NaOH. The free fatty acid content is expressed as acid degree (mls. of N NaOH per 100 gms. of fat).

EXPERIMENTAL RESULTS

Homogenized raw milk. In this phase of the study, selected raw milk was standardized to approximately 10 per cent fat by separation and this milk then divided into two portions. One portion served as the control and was non-homogenized, the other portion was homogenized at 700 pounds pressure and then stored at approximately 25° C. A sample of the homogenized milk was secured immediately after homogenization and at intervals thereafter (usually 15-minute intervals) until the milk had become definitely rancid. At specified intervals, samples, including the control, were

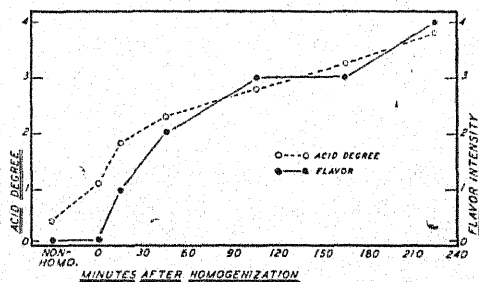


FIG. 1. Relationship between intensity of rancid flavor and increases in acid degree following homogenization of the milk at 700 pounds pressure.

quickly heated to 142–144° F. for 20 minutes and then cooled. After samples for the trial were obtained, portions of each were examined “blind” organoleptically, and other portions were used to supply the fat for titration. Seventeen trials were conducted and six to nine samples were examined for changes in flavor and fat acidity in each trial.

Average results of four trials in which the samples were secured at the same intervals before and following homogenization are illustrated in figure 1. This figure portrays (a) the general relationship between increase in acid degree and development of rancidity; (b) the fact that considerable increase in acid degree may occur before the rancid flavor is detectable but that rancidity develops within a few minutes after homogenization.

Homogenization increased the acid degree of the fat approximately three-fold, i.e., from 0.43 to 1.2, without producing a rancid flavor that was detectable by taste. Within 15 minutes the acid degree had increased to approximately 1.8 and the judges usually graded the flavor as “questionable” in regard to the rancid flavor. By the end of 30-minute and 45-minute periods the samples were usually rancid and the acid degree of the fat for the 45-minute period averaged approximately 2.3.

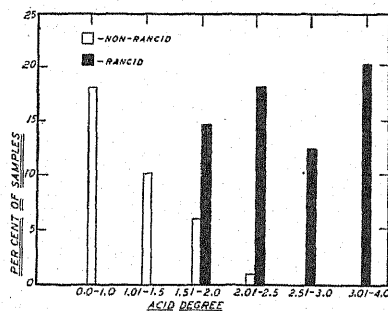


FIG. 2. Distribution of non-rancid and rancid samples of homogenized milk on basis of acid degree of the fat.

The relationship between acid degree and rancidity of all of the samples examined in the 17 trials is illustrated by figure 2. This graph reveals that the rancid flavor was generally detectable when the acid degree was within the range of 1.5 to 2.0. Below an acid degree of 1.5 no samples were criticized as being rancid whereas practically the reverse condition held when the acid degree was above 2.0. Of all the samples examined, approximately one-third were adjudged "non-rancid," with 28 per cent having acid values of 1.5 or less. Only one sample having an acid degree above 2.0 was graded non-rancid.

Fat from non-homogenized and homogenized raw milk: In the second phase of this portion of the study, fat of varying acidities was secured from non-homogenized and homogenized milk. This fat was then re-homogenized into pasteurized skimmilk in proportions to give a 5 per cent milk and the mixture examined organoleptically. Results of several typical trials are presented in table 1.

These data reveal that in no case was a rancid flavor produced in the fat-skimmilk mixture even though the acid degree of the fat ranged from 0.5 to 11.5. Instead, an oxidized, oily, or tallowy flavor resulted in the samples; this flavor appearing in low acid-degree samples as well as in the high, but to

TABLE 1

Effect on the flavor of the mixture when butter fat of varying acid degrees is homogenized into skim milk

	Trial 1		Trial 2		Trial 3	
	Acid degree of fat	Flavor of mixture	Acid degree of fat	Flavor of mixture	Acid degree of fat	Flavor of mixture
Skimmilk	OK	OK	OK
Skimmilk plus fat	0.5	Oxidized	0.5	Oxidized	0.5	Oxidized
Skimmilk plus fat	1.5	Oxidized	1.5	Oxidized	6.5	Oxidized
Skimmilk plus fat	2.5	Oxidized	2.5	Oxidized	0.6	Oxidized
Skimmilk plus fat	3.5	Oxidized	4.0	Oxidized	7.5	Oxidized
Skimmilk plus fat	4.3	Oxidized	0.5	Oxidized
Skimmilk plus fat	5.6	Oxidized	11.5	Oxidized

slightly less intensity. The degree of oxidized flavor was never sufficient to have obscured the rancid flavor.

Examination of the butteroil used in these trials for the rancid flavor also gave negative results, the oil possessing an oxidized flavor when the acid degree was high. However, when the acid degree was less than 1.0, the butteroil did not uniformly exhibit the oxidized flavor. Although the fat itself, following the removal of the curd and water by filtration, did not possess the rancid flavor, the buttermilk obtained by the churning of cream with a high acid degree was always intensely rancid.

DISCUSSION

The direct relationship between the development of rancidity in milk and the increase in acid degree of the fat as observed in this experiment is in agreement with the findings of Krukovsky and Herrington (7). However, the threshold value for the flavor is approximately twice as high in these studies on homogenized milk as was reported by Krukovsky and Herrington for normal milk. This difference may be explainable on the basis that when lipolysis is accelerated by homogenization, lipase activity proceeds at the expense of those fatty acids which contribute to the titratable acidity of the fat at a proportionately greater rate than is true in the case of non-homogenized milk.

The results secured in this study serve as a basis for explaining the differences which have been reported relative to the correlation between the acid degree of the fat and the rancid flavor. The lack of such a correlation in commercial butter observed by Fouts (1) is understandable in view of the fact that the rancid flavoring materials in the milk or cream are largely lost in the buttermilk when the fat is obtained by churning, even though the fat itself shows the presence of appreciable quantities of free fatty acids. Whether or not butter from rancid cream will retain the rancid flavor would appear to be dependent upon the amount of buttermilk incorporated into the butter, and thorough washing of the butter may completely wash away the rancid-flavored fatty acids. The author has observed that the churning of rancid cream did not uniformly result in rancid butter. This does not eliminate the possibility, however, of the butter becoming rancid on storage due to continued lipase activity. The problem of water solubility of the fatty acids responsible for the rancid flavor is considered more thoroughly in the subsequent paper.

The fact that samples of butteroil with high acid degree produced an oxidized or oily flavor when homogenized in skim milk does not necessarily mean that there is a relationship between free fatty acid content and the oxidized flavor under these conditions. Such a relationship was not apparent since fat of low acid degree also produced this flavor in the milk. The important point is that the rancid flavor was not produced by this process, even though the fat was obtained from rancid homogenized raw milk.

The apparent absence of at least a portion of the lower fatty acids from butteroil prepared by churning the cream and filtering the fat as indicated by the absence of the rancid flavor may tend to cast some doubt as to the reliability of the titration of the fat as a means of measuring lipolysis. It does appear that such titration values do not necessarily represent all of the fatty acids that are freed by lipase activity, although the results of Gould and Trout (2) would indicate, at least, almost complete measurement of these acids. To secure greater accuracy it may be desirable to utilize some other method than churning in obtaining the fat from the milk or cream. The author has made use of an extraction procedure and the results secured were encouraging. Higher fat titers were secured in the extracted fat than in the churned fat, but the possibility that these higher values may be due to the inclusion of other than fatty acids has not been eliminated.

CONCLUSIONS

Raw milk, homogenized at 700 pounds pressure, is usually rancid when the acid degree of the fat is within the range of 1.5 or 2.0.

Fat secured from rancid milk and with acid degrees as high as 11.5 did not itself possess a rancid flavor nor did it produce a rancid flavor when homogenized into pasteurized skimmilk.

Free fatty acids in butterfat which is obtained by churning are not responsible for the typical rancid flavor of dairy products.

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SOLUBILITY AND VOLATILITY OF FATTY ACIDS INVOLVED IN LIPOLYSIS IN HOMOGENIZED RAW MILK*

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Homogenization of raw milk, a process known to greatly accelerate lipase activity, results in immediate increase in the free fatty acid content of the fat with homogenization at 1500 pounds pressure increasing the free fatty acids approximately five-fold within a few minutes following processing (6). Since information relative to the characteristics of the fatty acids involved in this lipolysis is meager, experiments were conducted to ascertain their solubility and volatility. The results of these experiments are herein presented.

Steam distillation procedures have been used to study the volatility of free fatty acids in milk and milk products. Roahen and Sommer (9) used this procedure to measure the extent of lipolysis in milk and cream and Fouts (4) studied the volatility of free fatty acids in butteroil of varying acid degrees secured from butter by steam distillation of 10 grams of fat and collecting and titrating 200 ml. of distillate. He secured values for volatile fatty acids approximating 15 per cent of the total fat acidity. Davies (2) reports that volatile acids account for less than five per cent of the total titratable value of the fatty acids, whereas oleic acid is responsible for 60-70 per cent of this value.

Dyer (3), and Hiscox and Harrison (7), used steam distillation in the study of pure volatile organic acids. Hiscox and Harrison (7) steam distilled acetic, butyric, caproic, caprylic, capric, and lauric acids (acids neutralized with NaOH and then acidified to pH 2 with H_2SO_4) and secured complete recovery from water when distillate volume was "5 times original volume." Recovery of these acids from cheese was retarded, the retardation being especially marked for acetic, caprylic, caproic, and lauric acids. Butterfat and cheese fat also retarded distillation of the water-insoluble fat-soluble acids.

Methods of preparing samples of cheese for steam distillation were studied by Hiscox, Harrison, and Wolf (8). They found that a method involving warm water washing of the cheese to obtain the fat, ether extraction of the fat, washing of the fat with NaOH, acidifying and steam-distilling this rinse, resulted in volatile acid values which were about 4.5 times as high as those from direct steam distillation in the case of Stilton cheese and about twice as high in the case of Cheddar.

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PROCEDURE

The studies reported in this paper were conducted with two purposes in mind: (a) to determine the solubility of fatty acids in fat from homogenized raw milk by washing the fat either with water or with a weak alkaline solution; (b) to determine the volatile fatty acid content of the butterfat by direct steam distillation of the butterfat itself and by the distillation of the alkaline rinsings of the butterfat.

Butterfat with high free fatty acid content was produced by homogenization of raw milk at 37° C. and at 500 pounds pressure. The butterfat was secured by churning, melting, centrifuging, and filtering.

Fat acidity was determined by the official procedure (1) with exception that a 10-gram sample, 25 ml. ethyl alcohol, and N/20 NaOH solutions were used. The free fatty acid content is expressed as acid degree (mls. of N NaOH per 100 gms. of fat).

A multiple steam distillation apparatus, capable of distilling four samples at once, was utilized for the studies on volatile acids. This apparatus consisted of a sodium chloride bath maintained at a temperature of 102–104° C. The bath was of sufficient size to accommodate four one-liter round bottom flasks containing the samples. Steam was generated in two five-liter round bottom flasks, each flask supplying steam for two samples. The steam entered the sample through Folin-type ammonia tubes with perforated bulbs at the bottom, and this inlet tube extended well below the surface of the samples. A Kjeldahl connecting bulb was placed at the outlet from the flask to insure against entrainment of the sample. Condensation of the steam enroute to and from the sample flask was reduced to a minimum by wrapping the conducting glass tubes with asbestos. By regulation of the temperature of the bath and the rate of steam it was possible to maintain fairly constant volume in the flasks containing the samples.

The volatile material leaving each sample passed to a Liebig condenser where it was condensed. The distillate then passed through a No. 40 Whatman filter paper to remove the water insoluble acids and into a 200-ml. volumetric flask. Titrations were conducted in each 200 ml. sample with 0.05 N NaOH, using phenolphthalein as the indicator. After each 200 mls. of distillate were secured, the condensers and the filters were washed with ethyl alcohol to dissolve the alcohol-soluble fatty acids. This alcohol fraction was titrated and the titer added to the values secured for the 200 mls. of distillate.

Blank distillations were made on water at pH 2. The blank values, which included those for the alcohol used to rinse down the condensers, averaged approximately 0.35 ml. of 0.05 N NaOH per 200 mls. of distillate.

RESULTS

Solubility of free fatty acids. In other work (5) the senior author observed that butterfat, secured from rancid cream by churning and filtering,

and with a high free fatty acid content did not possess a rancid flavor. This observation raised the question as to the water solubility of these fatty acids and trials were conducted to ascertain their solubility by washing the butterfat with warm water. In this experiment, approximately 20 gms. of butterfat having a high content of free fatty acids (acid degree approximately 5) were placed in a separatory funnel and shaken for 30 seconds with 20 ml. of water at 130-135° F. The fat layer was then permitted to rise and the aqueous layer removed and titrated. This washing was repeated six times. After the final washing, the fat was removed, dried, and titrated. Results of the original and final titrations on the fat are shown in table 1.

These results show no decrease in the acid degree of the fat to occur with six washings with warm water. Although the data are not shown, titrations of the washings revealed no measurable quantity of fatty acids were contained therein. These findings indicate either that the water-soluble fatty acids are not retained by the fat during its churning and purification or that they have greater affinity for the water than for the fat.

TABLE 1
Influence of washing butterfat with water on the acid degree

	Trial 1	Trial 2	Trial 3	Trial 4
Original acid degree of fat	4.93	4.87	4.82	4.80
Final acid* degree of fat	4.91	4.88	4.85	4.85

* Following washing of 20 grams of fat with six successive portions of warm water.

Other trials were conducted to determine the efficiency with which a weak alkaline solution may remove the fatty acids from fat. The method used was similar to that proposed by Hiseox *et al.* (6) for cheese. Fat samples weighing either 25 or 50 grams were dissolved in 100-120 mls. ethyl ether in a separatory funnel. Twenty-ml. portions of 0.05 N NaOH were added and the mixture shaken gently with rotary motion to avoid emulsification. The aqueous layer was permitted to settle and was drained away. The fat-ether mixture was then washed with 20-ml. portions of distilled water, the ether evaporated on a hot plate and in a 100° C. oven, and the fat titrated for acidity. Results are presented in table 2.

These data reveal that this procedure of removing fatty acid is highly efficient, with three 20-ml. portions of 0.05 N NaOH being sufficient to reduce the fat titer by more than 90 per cent in a 50-gram sample when the acid degree approximates 3 to 5. However, there appears to be a point of irreducible minimum in the fat acidity and additional washings do not appreciably alter this value. This residual acidity amounts to approximately 0.15-0.30 ml. of 0.05 N NaOH per 10-gm. sample of fat. These values may be due to a slight degree of hydrolysis of the fat or a slight extent of end-point fading occurring during the titration procedure. On the basis of

these results it would appear that three to five washings of fat with small portions of weak alkaline solutions, followed by two rinsings with water, will remove practically all of the free fatty acids, provided that the alkali used is in excess to that required for neutralization by at least 75 per cent. This amount of washing is decidedly less than was used by Hiscox, Harrison, and Wolf (7) for cheese fat.

Steam distillation of fatty acids. Steam distillation of fatty acids was conducted using the multiple unit described under "Procedure." In preliminary trials, butyric acid (Eastman) was used for the distillation. Suffi-

TABLE 2

Efficiency of removing fatty acids from a fat-ether mixture by washing with a weak NaOH solution

Trial No.	Size of sample	Amount of ether	Amount of*		Titer of fatt†			Volume alkali times volume of fat	Excess of 0.05 N NaOH solution
			Washing with 0.05 N NaOH	Rinsing with water	Original	Final	Reduction		
	<i>gms.</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>			%		<i>ml.</i>
1	50	120	2-20	2-20	7.47	0.83	88.88	0.8	2.65
2	50	120	3-20	2-20	7.46	0.45	93.97	1.2	22.70
3	50	120	4-20	2-20	7.46	0.30	95.98	1.6	42.70
4	50	120	4-20	2-20	6.90	0.23	96.67	1.6	45.50
5	50	120	5-20	2-20	6.90	0.25	96.38	2.0	65.50
6	50	120	5-20	1-20, 2-10	6.90	0.15	97.83	2.0	65.50
7	50	120	6-20	2-20	6.90	0.30	95.65	2.4	85.50
8	50	120	6-20	2-20	6.90	0.20	97.10	2.4	85.50
9	50	120	7-20	2-20	8.60	0.20	97.68	2.8	97.00
10	25	100	8-20	2-20	8.45	0.23	97.28	6.4	138.87
11	25	100	4-20	4-20	8.45	0.20	97.63	3.2	58.87
12	25	120	5-20	4-20	8.45	0.18	97.87	4.0	78.87
13	25	120	4-10	4-10	8.45	0.23	97.28	1.6	18.87
14	25	120	5-10	4-10	8.53	0.25	97.07	2.0	28.67

* First values indicate number of washings, second values indicate the volume used per washing; thus 2-20 indicates 2 washings of 20 ml. each.

† Titer of fat represents mls. 0.05 N NaOH solution per 10 gms. To change to acid degree divide by 2.

cient butyric acid was used to require 20-25 ml. of 0.05 N NaOH for neutralization. This acid was neutralized with the alkali, then adjusted to pH 2 with H_2SO_4 . The volume was standardized to 200 ml. and steam distillation was conducted. Each 100 ml. of distillate was titrated and corrected for the blank value. Average results secured for seven trials are presented in figure 1, in which a comparison is made with the results of Hiscox and Harrison (7). The two curves exhibit similar trends and indicate that when the volume of distillate is twice the original volume 98 per cent of the acid is recovered. These data also are in agreement with those secured by Dyer (3). Although results are not presented, distillation of n-caprylic acid revealed even more rapid recovery, with approximately 93 per cent being recovered

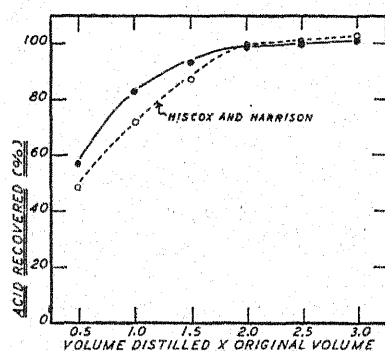


FIG. 1. Rate of steam distillation of n-butyric acid in comparison to rate observed by Hiscox and Harrison.

with distillation of one times the original volume. This compares favorably with the 95 per cent reported by Hiscox and Harrison (7).

Following the standardization of the distillation procedure by use of the pure fatty acids, trials were conducted in which butterfat having high free fatty acid content was steam distilled. In some trials, approximately 150 grams of butteroil were used, in other trials, the size of the sample was approximately 50 grams. The volume of the sample was increased to 200 ml. with acidified water and 200 ml. portions of the distillate were titrated. Results of 7 trials are presented in table 3.

Data in this table reveal that steam distillation of the pure butteroil results in poor recovery of the fatty acids. This may result due to the fact that no appreciable quantity of the lower volatile fatty acids are in the free state, or to the retardation action of the fat. The volatility of 4-6 per cent of the fatty acids is considerably lower than 15 per cent reported by Fouts (4). However, Fouts titrated 200 ml. of distillate from 10 grams of fat, a value representing 20 times the original volume as contrasted to 6 to 10 times the original volume used in the experiments herein reported.

TABLE 3

Amount of volatile fatty acids recovered by steam distillation of butterfat with high free fatty acid content

Sample No.	Acid degree of fat	Per cent of total free fatty acid after distilling		
		3 × original vol.	6 × original vol.	10 × original vol.
1	3.62	2.43	5.03
2	4.25	2.10	3.14	4.02
3	4.25	2.28	3.38	4.34
4	4.35	1.84	2.73	3.96
5	4.33	2.35	3.34	4.50
6	6.75	2.35	5.62
7	7.00	2.86	6.12

To study further the volatility of free fatty acids in butterfat, butterfat having previously undergone considerably lipolysis was divided into two lots. One lot was steam-distilled directly. The other lot was dissolved in ether, washed with 0.05 N NaOH solution and then rinsed with distilled water to remove the remainder of the salts. Fifty grams of fat were used, washed with six 20-ml. portions of the alkali, and rinsed with two 20-ml. portions of water. The alkaline washings and the water rinse were combined, adjusted to pH 2 with H_2SO_4 and the solution distilled. Two-hundred-ml. distillate portions obtained from the fat and from the alkaline rinsings of the fat were titrated. Acid degrees of the fat used in this study ranged from 3.62 to 7.0. Average results are portrayed in figure 2.

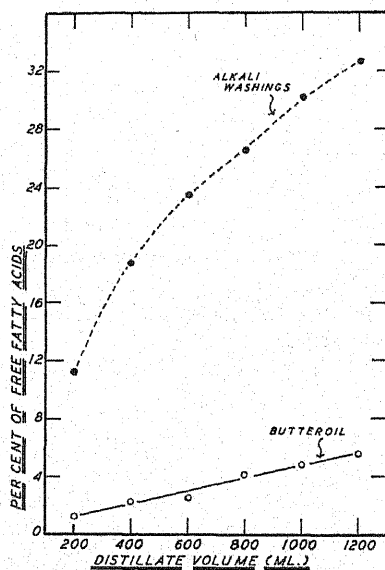


FIG. 2. Recovery of volatile fatty acids from butteroil having high acid degree when steam distillation was conducted on the butteroil and on the alkali washings from the butteroil. (Original volume—200 ml.)

This figure illustrates the remarkable difference in volatility of fatty acid when the acids are removed from the fat and then distilled and when they are distilled directly from the fat. Distillation of six times the original volume resulted in a recovery of only 5.7 per cent of the acids when the distillation involved the fat itself, whereas a recovery of 32.7 per cent resulted when the fatty acids were distilled separately. The rate of distillation of the fatty acids from the acidified-alkaline rinse is also interesting. Approximately 30 per cent of the fatty acids recovered during the total distillation period were recovered by distillation of one times the original volume. However, an appreciable quantity of acids were being distilled at the close of the distillation.

Results secured with the acidified-alkaline rinse indicate that a higher percentage recovery of fatty acids may be obtained from lower acid degree fat. In one trial in which the fat possessed an acid degree of 3.62, 48.8 per cent of the acids were recovered by distillation of seven times the volume, whereas under similar conditions, fat with an acid degree of 7 permitted a recovery of 31.4 per cent. However, additional information is needed in this connection before conclusions are drawn.

DISCUSSION

The failure of the washing of the fat with successive portions of warm water to reduce the fatty acid content indicates the possibility that water-soluble fatty acids either are not retained in any appreciable quantity by the fat or are held in such a manner that they are not removed by the water-washing procedure. These findings demonstrate that washing of the butter granules following churning and during the preparation of butteroil for fat titrations will likely have no influence on the resulting titer of the fat. Thus, the preparation of butteroil in lipolytic studies may be greatly expedited by utilizing water to remove the milk-solids-not-fat.

The steam-distillation studies show the undesirability of attempting to measure lipase activity in the milk or cream by steam-distillation of the fat. The proportion of volatile acids obtained by this procedure is so small that differences between samples may not be clearly demonstrated. In contrast, the steam-distillation of acidified-alkaline washings of fat appears to be an excellent method of studying lipolysis. Recovery of the acids was approximately five times as great for the alkaline washings as for the butteroil when the volume of the distillate was six times the original volume.

The high proportion of volatile acids secured when the acids were distilled separately from the fat may serve to indicate that the fatty acids involved are of the water-insoluble fat-soluble type. Hiscox and Harrison (7) found butterfat to distinctly retard the distillation of this type of acid, with the retardation increasing with increasing solubility of the acids in fat. This fat retention effect is especially marked in the results presented herein. In addition, the fact that these acids are not removed by washing fat with water and that the rancid flavor is not retained by the fat also indicates that the free fatty acids present in churned fat are of the fat-soluble type.

CONCLUSIONS

The fat acidity of butterfat from homogenized raw milk was not appreciably affected by washing with six successive portions of warm water. However, washing of an ether solution of the fat with a weak alkaline solution is an efficient method of removing the free fatty acids.

Steam distillation of butterfat is highly inefficient as a means of recovering volatile fatty acids, resulting in a recovery of approximately 5 per cent

when distillate volume was six times original volume. In contrast, distillation of acidified-alkaline washings of fat resulted in a recovery of approximately 32 per cent of the free fatty acids.

Free fatty acids in butterfat secured from homogenized raw milk by churning are apparently of the water-insoluble fat-soluble type.

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THE INFLUENCE OF LOW ENVIRONMENTAL TEMPERATURES ON INTRAMAMMARY TEMPERATURES*

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Since temperature plays an important part in all physiological processes it is only consistent to expect that variations in the temperature of the udder should affect the production of milk. Although the rectal temperature of the normal cow lies between 100° and 103° F., one would expect the udder, with its comparatively large exposed surface, to be influenced to some extent by the environmental temperature. Many reports have been published dealing with the effect on milk production of low environmental temperatures but no information regarding intramammary temperatures has been found.

Since the teats and lower portion of the udder are more exposed than other portions, one would expect temperature changes in this area to be most marked. Therefore, for the most part, only data on the temperature in the teat and the milk cistern have been used in this study.

Two methods of obtaining the intramammary temperature were used. Inserting a small metal thermometer (Model 226L001, Weston Electric Instrument Company) directly into the teat cistern and making readings as soon as a constant temperature was indicated, was one. Hereafter, this method will be designated as the "direct method." The other method, an "indirect" one, was that of estimating the intramammary temperatures from the temperatures of the first drawn milk.

The direct method of obtaining the intramammary temperature is unquestionably the better method. However, due to the danger of introducing infection into the udder, only cows which were to be discarded for milk purposes could be used in this manner. Of the eight cows on which direct temperature readings were obtained, four were reported by our veterinary department to be mastitic in one or more quarters. However, none of these cows had acute mastitis in the commonly accepted meaning of the term.

Cows with mastitis were kept in a separate barn from the one housing the healthy animals. The removal from the herd at frequent intervals of all cows infected with mastitis as well as the difficulty of carrying out even two trials at the same environmental temperature made it impossible to secure adequate data by the direct method. For these reasons it seemed wise to check the direct method by an indirect method and then use the indirect method to obtain more data from cows free of mastitis.

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¹ Mario Cornejo, D.V.M., Universidad de Chile, uses the customary form in Chile of placing the first letter of his mother's surname after his surname.

During the exposure period, the cows were confined to a small protected yard just south of the barn. Some of the differences noted with the same cow and the same environmental temperature may have been due to differences in activity of the cows from day to day and to wind velocity. Also, when the sun was shining, the environmental temperature tended to fluctuate rapidly. Data showing the effect of repeated sampling, *et cetera*, were obtained in the barn proper after the severe winter weather had passed.

Before securing direct readings each cow was brought into a room whose temperature varied from 65° to 75° F. The udder was washed and disinfected. The thermometer whose stem was seven and three-fourths inches long, was inserted as far as possible into the mammary gland and readings made by means of a mirror held beneath the inverted dial. The dials were graduated in two degree intervals and the thermometers were guaranteed by the makers to be accurate to within one-half of one per cent, which was verified. The order in which readings were taken is indicated in the tables.

In making indirect readings, the udders were quickly washed with chlorine solution and samples collected as soon afterwards as possible. The samples of milk were collected in four test tubes, one for each quarter of the udder. These 60-ml. test tubes were each inserted into pint thermos bottles and securely attached so as to be doubly insulated from the effects of the temperature of the room in which readings were made. Cork stoppers with small thermometers (like those commonly used in the Van Slyke blood gas apparatus) completed the assembly.

Just before milk samples were obtained the thermos bottles were removed from the constant temperature oven and brought to the room where temperature readings were made. Each 60-ml. tube was emptied of water, filled at once with warm milk, preferably from a cow subjected to the same temperature changes as the environmental animals, and the tubes corked. In taking milk samples from experimental animals, the "tempering milk" was discarded and each tube filled at once with milk from a quarter of the udder of an experimental animal. The thermos bottles were held close to the end of the teat and were filled with milk as rapidly as possible. Due to foaming only about 50 ml. of milk were removed at each sampling. The tubes were then corked and the thermos bottles inverted so that milk and not foam was sure to cover the thermometer bulbs. Readings were made as soon as samples from all quarters had been obtained.

RESULTS

Before attempting to study the effect of low environmental temperatures on the udder of the cow it seemed wise to determine the normal range of udder temperatures. Table 1 not only shows the udder temperatures by the direct and indirect method, but data are also presented on temperatures of each quarter both for healthy cows and those considered to be carriers of

mastitis. These values were obtained after the cows had been in the barn for several hours. Although barn temperatures varied from 58° to 70° F. at different times during the experiment, readings were obtained for all quarters of the udder at any one temperature.

With some cows the temperature of the milk in the quarter sampled last was higher than the milk in the quarter sampled first. This was not consistently true. There was a definite tendency, though, for the milk in the two front quarters to be slightly higher in temperature than the milk in the two rear quarters. There was no regular difference between the intramam-

TABLE 1

Data showing average intramammary temperature of udder with environmental temperatures of 58° to 70° F.

Quarters (observations made in order indicated)	No. of observations	Direct method*	No. of observations	Indirect method†
Cows free of mastitis				
		° F.		° F.
1. Right front	10	99.1	28	97.9
2. Right rear	10	98.7	28	97.6
3. Left rear	10	98.7	28	97.4
4. Left front	10	99.1	28	97.8
Two front	99.1	97.85
Two rear	98.7	97.5
Cows with mastitis				
1. Right front	31	99.0	30	97.6
2. Right rear	31	98.7	30	97.9
3. Left rear	31	98.5	30	97.2
4. Left front	31	99.0	30	97.6
Two front	99.0	97.6
Two rear	98.6	97.5

* Thermometer inserted into gland cistern.

† Temperature of first drawn milk.

mary temperatures of cows free of mastitis and those known to have been previously infected with mastitis.

The intramammary temperatures secured by the indirect method are about 1.2 degrees lower than those obtained by the direct method. These differences were greater when barn temperatures were low and with cows which milked slowly. The intramammary temperature, as obtained by the direct method, showed no consistent change with the usual fluctuations in barn temperature (58° to 70° F.), so it is apparent that the indirect method becomes less reliable as lower environmental temperatures are studied.

Variations of the intramammary temperature for the same cow varied between trials as much as 1° F. by the direct method and 1.5° F. by the indirect method. Changes in environmental and body temperature caused some of this fluctuation. Washing the udder before collecting the samples

may have produced different responses on different days. Differences in the amount of bedding protecting the udder from the barn floor also varied from day to day and may have caused some minor fluctuations in intramammary temperature.

Although more pendulous udders might be expected to show lower temperatures than the so-called "tight vessel" type, no definite trend was evident at barn temperatures.

The data presented in table 1 have been rearranged in table 2 so as to show whether the breed or the milk volume of the udder influenced the intramammary temperature. No evidence was found to indicate that either factor is important when the environmental temperature is between 58° and 70° F.

TABLE 2
Effect of milk production and breed on intramammary temperature (indirect method)

No. and breed of cows in each trial	No. trials	Average daily production			
		> 60 lbs.	25-60 lbs.	< 25 lbs.	All cows
		Temperature*	Temperature*	Temperature*	Temperature*
20 Holstein	3	98.2	97.9	98.0
6 Guernsey	3	98.0	98.3	98.1

* Temperature of first drawn milk. Environmental temperature (barn temperature) varied slightly but samples from every cow were taken at each trial.

TABLE 3
*Temperatures of consecutive samples of milk obtained from the same udder**

Cows used in each trial	Trials	Average temperature of milk from all quarters		
		First sample	Second sample	Third sample
No.	No.	° F.	° F.	° F.
20	3	98.9	98.9	99.1
3	1	98.1	98.7	99.2

* Data obtained during each trial were taken at the same environmental temperature. The environmental temperature between trials varied from 58-68° F.

In trials, results of which are reported in table 3, a second series of milk samples was taken as soon as the temperatures on the first series of samples had been noted. And then a third series of samples was taken from the same cow, all within about a three-minute interval. During this interval most of the cows had begun to "let down" their milk. Although the last series of samples gave higher values than the first two, the temperature differences were not very important.

Only a very small amount of data bearing on the importance of this "letting down" process were obtained with cows exposed to low environmental temperatures. By the indirect method, readings were obtained on four cows after three hours' exposure at 20° F. (table 4). The udders of

TABLE 4

Effect of handling the udder on the temperature of the first drawn milk obtained from cows exposed to an environmental temperature of 20° F. for 3 hours

	Front quarters in °F.	Rear quarters in °F.	All quarters in °F.
Cow No. 1701			
Udder not handled before sample was taken	95.9	95.4	95.6
Cow No. 1044			
Udder washed before sample was taken	97.2	96.8	97.0
Cow No. 1751			
Udder not handled before sample was taken	94.1	92.6	93.7
Cow No. 1756			
Udder washed before sample was taken	97.4	96.7	97.0

two of these cows were washed and disinfected as already noted in the methods of procedure. With the other two cows, milk samples were withdrawn without previous handling of the udder. Although there were always differences in intramammary temperature between cows exposed to the same environmental temperature, the striking differences noted in table 4 would indicate that the handling of the udder is likely to cause the cows to "let down" their milk and that this phenomenon explains why the milk in the udders of these cows tends to "heat up," probably by adding more milk from higher up in the gland to that already present in the milk cistern.

TABLE 5

Relation between rectal temperature and intramammary temperature when the environmental temperature varied from 58° to 70° F.

Rectal temperature in °F.†	No. of cows	Total No. of observations	Average intramammary temperature*		
			Fore quarters in °F.	Rear quarters in °F.	All quarters in °F.
100.8	4	24	99.1	98.9	99.0
101.0	3	9	98.7	98.7	98.7
101.1	1	1	98.7	98.8	98.7
101.2	5	25	98.5	98.6	98.6
101.3	3	6	99.2	98.9	99.0
101.4	5	30	99.0	98.8	98.9
101.5	3	12	98.0	97.2	97.6
101.6	4	12	97.8	97.6	97.7
101.7	2	2	97.7	97.3	97.5
101.8	2	4	98.3	98.3	97.3
101.9	3	6	97.7	97.3	97.5
102.0	2	4	98.1	97.9	98.0
102.1	1	1	97.7	97.3	97.5
102.2	1	1	99.4	99.2	99.3
103.1	1	1	99.4	100.0	99.7
103.2	1	1	100.4	100.3	100.4

* Indirect method.

† Rectal thermometer with extended shank was used so that bulb was inserted two inches deeper than normally.

TABLE 6

Relation between environmental temperature and intramammary temperature

Environmental temperature in °F.	No. of cows	Total No. of trials	Temperature in °F.			
			Fore quarters	Rear quarters	Average	Rectal
Direct method						
-14	2	1	97.1	96.6	96.9	101.5
-12	1	1	96.2	96.0	96.1	101.4
-10	2	1	95.8	95.3	95.5	101.0
9	2	1	98.2	97.2	97.7	101.3
14	2	2	97.9	96.9	97.3	101.7
16	2	1	98.3	98.0	98.1	101.9
17	2	1	98.3	97.6	97.8	101.6
18	1	2	98.2	97.7	97.9	102.0
19	1	1	98.6	97.7	98.1	101.9
20	2	3	98.0	97.6	97.8	101.7
28	3	2	98.6	97.9	98.2	101.5
29	3	1	98.8	98.8	98.8	101.5
30	2	1	98.7	98.1	98.4	101.6
31	5	1	98.7	97.8	98.2	101.7
32	1	1	98.7	98.3	98.5	101.8
40	1	2	99.0	98.9	98.9	101.7
44	3	1	98.6	97.0	98.1	101.9
48	3	1	98.5	98.3	98.4	101.7
49	2	1	99.2	98.7	98.9	101.3
52	1	2	99.3	99.0	99.1	101.9
54	2	1	99.1	98.7	98.9	101.6
55	2	1	99.0	98.6	98.8	101.4
56	1	1	99.3	99.0	99.1	101.3
58	3	1	99.0	98.6	98.8	101.9
60	2	1	98.8	98.5	98.7	101.6
61	2	1	99.0	98.9	99.0	101.5
65	2	1	99.0	98.5	98.8	101.4
Indirect method						
14	1	1	98.3	97.3	97.8	101.1
16	4	1	96.7	97.6	96.2	101.5
17	3	1	96.4	95.7	96.0	101.6
18	6	1	96.2	95.4	95.8	101.9
19	3	1	96.9	95.8	96.2	101.3
20	4	3	96.7	95.5	96.1	100.9
28	5	2	97.6	97.1	97.3	101.5
29	3	1	97.7	97.5	97.7	101.5
30	4	2	97.7	97.1	96.3	102.1
31	2	1	97.1	96.7	96.9	101.8
32	3	2	97.1	96.3	96.7	101.8
35	5	1	97.5	97.3	97.4	101.4
40	4	2	97.7	97.1	97.4	101.7
48	1	1	97.2	97.0	97.1	101.9
49	1	1	97.9	97.3	97.6	101.6
52	4	2	97.5	97.0	97.2	101.7
56	3	1	97.5	97.2	97.3	101.8
57	6	1	98.8	98.7	98.7	101.3
58	14	1	99.0	98.9	98.9	101.3
68	9	1	99.1	99.3	99.2	101.2
70	21	1	98.6	98.4	98.5	101.4

Since rectal temperatures were always taken at the same time as the temperatures of the udders were being determined, it seemed wise to see if there was any relationship between them. These data, shown in table 5, indicate no relationship between rectal temperature and udder temperature except when the rectal temperature rose above 103°F . The samples of milk secured from cows with a high rectal temperature showed a slightly higher temperature than milk from the rest of the herd tested.

The average differences between the rectal and udder temperatures during the winter was 3.6°F ., by the indirect method, and 2.7°F ., by the direct

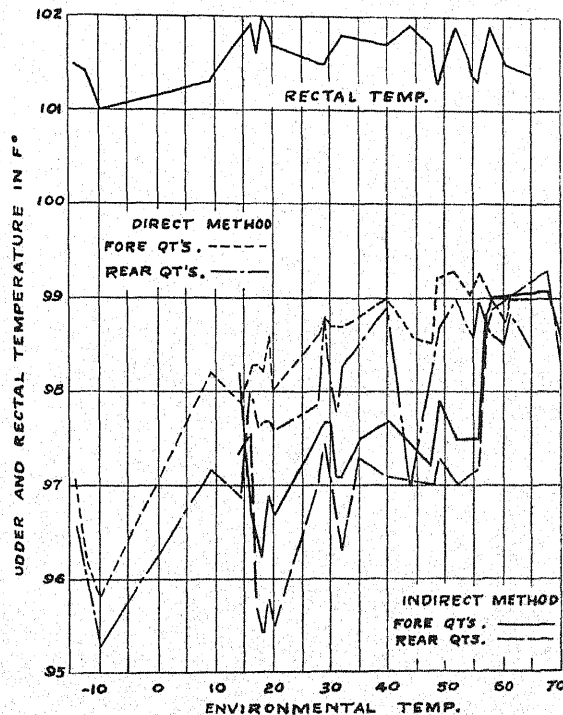


FIG. 1. Relation between environmental, body (rectal) and udder temperatures.

method. The four cows which had a high rectal temperature showed an average difference between rectal and intramammary temperature when measured by the indirect method of only 3.1°F .

Most of the information presented so far was obtained with cows housed in a reasonably warm, well-constructed barn. These data give us some idea of the general range of variability in intramammary temperature which may be expected under a moderate environmental temperature. Data are presented in table 6 which show the effect on the udder of external temperatures ranging from 70° to -14°F . A total of thirty cows were used in twenty-six trials to obtain this information. Nineteen of these cows were free from mastitis while eleven of them were, or had been, infected with mastitis. The

time of exposure was never less than one hour when direct temperature readings were taken and never less than two hours when indirect readings of udder temperatures were taken. Most of the cows were left outside longer periods unless the weather was very severe.

From the data presented in table 6 and figure 1 it is evident that the intramammary temperature is influenced by low environmental temperatures. During the coldest weather the intramammary temperature was over 5° F. below the rectal temperature when recorded by the direct method. This should be compared with a difference of 2° to 3° F. when the environmental temperature varied between 58° and 70° F. If the cows had been kept quiet and exposed to sub-zero weather, it is quite probable that the difference between the temperature in the teat cistern and in the lower part of the gland cistern, and the rectal temperature, may actually have been as great as 10° F. This is especially true if the animal had lain on the cold ground or snow. The difference in temperature between the fore and rear quarters also became greater as the temperature dropped, probably because of the greater surface exposure of the rear quarters. Differences in rectal temperature as a result of exposure were negligible.

Only one observation was made with a dry cow. This animal was left out of doors for an hour when the temperature was 10° below zero. By the direct method, temperatures of the different quarters were as follows: right front, 96° F., right rear, 93° F., left front, 94° F., and the left rear, 91.9° F. The unusually low readings for the rear quarters may have been caused by obstructions which would not allow the thermometer to be inserted farther than the base of each teat. This cow's udder was very pendulous and the teats large. In spite of this condition she never appeared to suffer from exposure while cows which were milking heavily occasionally had the ends of their teats frosted. In fact, the danger from low temperatures appeared greatest with cows whose udders were full of milk.

CONCLUSIONS

When cows were kept in a barn where the temperature varied between 58° and 70° F., the temperatures of their udders (teats and milk cisterns) averaged 2.5° to 3.0° F. lower than their rectal temperature. When cows were exposed to sub-zero weather the observed differences increased to around 5° F. The movement of the cows into the barn and the washing of the udder before inserting the thermometer into the gland cistern decreased the actual difference between rectal and udder temperatures. Otherwise, greater differences would have been observed.

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THE COMPARATIVE EFFECTS OF CONTINUAL AND ROTATIONAL SYSTEMS OF GRAZING ON THE CAROTENE CONTENT OF PERMANENT PASTURE HERBAGE AND OF THE MILK PRODUCED THEREFROM*

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The favorable returns from intensive systems of pasture management in Europe have attracted widespread attention in this country. One of the foremost advantages affirmed for this system is the improvement of the quality of the herbage. Since improved pastures are generally accepted as a practical solution to many difficult nutritional problems in the livestock industry today, any practical measure that reputedly enhances the nutritive value of the pasture plants merits consideration.

Rotational grazing, an essential phase of intensive pasture management, has been reported to increase the carrying capacity of pastures in several cases (9, 10, 19). Conceivably this increased yield could be related to qualitative changes, particularly in carotene values, of the herbage. Since there is a paucity of information on this subject, the need was deemed adequate to warrant investigation.

Thus a determination of the comparative effects of continual and rotational systems of grazing on the carotene of the herbage, primarily, and of the milk, secondarily, constituted a supplementary phase to a three-year study designed to ascertain the applicability of rotational grazing to improved Bermuda grass pastures.

EXPERIMENTAL

Pastures. The grazing areas were improved pastures, previously described (5). The total area of six acres was divided by permanent fences into three two-acre paddocks. Each year one of these was continually grazed while the remaining two, subdivided by temporary electric fences to form a four-unit system, were rotationally grazed. In the course of the three-year investigation each paddock was grazed continually one season and rotationally two seasons.

The flora consisted primarily of hop clover (*Trifolium procumbens* L.) in early spring and Bermuda grass (*Cynodon dactylon* (L.) Persoon) during the remainder of the grazing season. However, small amounts of other

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plants including white Dutch clover (*Trifolium repens* L.), bur clover (*Medicago arasica*) and common vetch (*Vicia sativa*) also contributed to the early grazing. In late spring during the brief interim between the maturation of the legumes and the development of Bermuda grass to the grazing stage and in the latter part of summer when droughts occurred, available herbage was sometimes sparse.

Annual applications of a 6-12-6 fertilizer were made at the rate of 400 pounds per acre during either late winter or early spring.

At various times throughout the grazing season, the paddocks were mowed to remove ungrazed clumps of grass and to check the growth of scattered areas of objectionable weeds.

Grazing animals. Lactating cows of the Holstein, the Guernsey and the Jersey breeds were used in comparing continual and rotational grazing. Recommended procedures (1) were followed in the selection, allotment and management of the cows.

The pasture grass was supplemented with a simple low-carotene concentrate mixture, which was fed according to the quantity of herbage available and the amount of milk produced by the individual cows. During periods of scanty grazing, beet pulp also was included in barn feeding.

The frequency of transfer from one unit to another in the rotational system varied with the stage of maturity and the rate of growth of the pasture herbage. Hence, the rotational rate was more rapid in spring than in the latter part of the pasture season. During the annual grazing period, an average of six rotation cycles of 29.5 days each were completed. In a representative cycle each pasture unit was grazed 7.5 days, leaving an interim of 22 days for recovery.

Herbage samples. The samples of pasture herbage were composites representing clippings collected at random over the respective pasture areas. The plants were cut at a height approximating the level to which it was grazed by the cows. In the rotational system a sample was taken from each section immediately before the cattle were released onto the area. In the continual system the first sample was gathered shortly preceding the initial grazing, and subsequent samples were collected at the same time as each alternate sample from the rotational series was clipped. This plan resulted in the collection of approximately two samples from the continually grazed area and four from the rotational sections each month; thus the number of samples from the two areas was in the same ratio as the acreage involved.

Each gross sample of collected grass was chopped sufficiently fine to permit accurate sub-sampling for carotene analysis.

The analytical procedure for the determination of carotene was based on the principles set forth by the Association of Official Agricultural Chemists (2) for the preliminary extraction and the modification described by Hegsted *et al.* (7) for the final extraction. The amount of extracted caro-

tene was calculated from colorimetric measurements, using a standardized solution of potassium dichromate as the reference reagent.

Milk samples. Individual milk samples for carotene analysis were collected from a representative of each of the three aforementioned breeds in the respective grazing groups. These samples were collected bi-weekly during three consecutive periods, pre-grazing, grazing and post-grazing. The minimum length of the first and last periods was one month, during which time the cattle were barn-fed, receiving corn silage as the only roughage. The duration of the grazing period varied from year to year, depending primarily on climatic conditions.

Each milk sample was a composite of aliquot portions taken from each of the three normal milkings during a day. In the period between milkings the collected milk was stored at a temperature of 35° to 40° F. Within three hours after the final collection, the sample was composited and subjected to the initial analytical procedures.

The fat of the milk was determined by the standard Babcock method and the carotene was measured by a procedure developed by one of the authors (Mitchell) in 1936. The available methods at that time required the use of milk fat as the starting material. These procedures necessitated not only the use of large volumes of milk but also the expenditure of much time and labor to prepare the fat. In searching for a suitable procedure the Roesse-Gottlieb (2) method of extracting fat from whole milk was adopted as the base from which the following procedure evolved:

A 50-ml. portion of milk is placed into a 250-ml. separatory funnel. Seven ml. of ammonium hydroxide and 50 ml. of ethyl alcohol (aldehyde-free) are added successively. The contents of the funnel are shaken after each addition. Fifty ml. of ethyl ether and 50 ml. of petroleum ether (b.p. 35-60° C.) are then added in the order listed and mixed gently after each addition. Vigorous agitation may result in emulsification and poor separation of the ether layer. The contents of the funnel are allowed to stand 30 minutes for complete separation after which the lower layer is drawn off and the remaining material reextracted with a mixture of 25 ml. of ethyl ether and 25 ml. of Skelly solve B (petroleum ether, b.p. 60-70° C.). All material from the two extractions is placed into a distillation flask and distilled under reduced pressure at a temperature of 70-80° C. to remove the more volatile solvents. Usually 10 to 15 ml. of a solution, mainly Skelly solve, is left in the flask.

This residual material is transferred to an Erlenmeyer flask where it is mixed with 25 ml. of an alcoholic potassium hydroxide solution (60 gm. potassium hydroxide made up to one l. with ethyl alcohol). The fat present is saponified by refluxing 30 minutes, after which the contents of the flask are cooled and transferred to a separatory funnel by rinsing with 20 ml. of water. The material is extracted twice with 20-ml. portions of Skelly

solve B. The extracts are combined and washed three times with small quantities of distilled water. This washed ether extract is concentrated under partial vacuum to less than 25 ml., after which it is dried over anhydrous sodium sulphate. The extract is then transferred to a glass-stoppered graduated cylinder. Hexane is added to make the volume up to approximately 25 ml., the dilution depending on the intensity of the color needed for comparison.

The carotene content of this solution is determined by means of a spectrophotometer.

Since this procedure was developed, other investigators (13, 17) have employed the Roese-Gottlieb principal of extraction in determining the carotene of milk and milk products.

Calculations. The quantity of carotene in the pasture herbage was calculated as micrograms per gram of dry matter and that for milk, standardized at four-per-cent fat, as micrograms per liter. Since this four-per-cent standard is frequently used in dairy calculations, it was adopted as the common base for expressing the carotene content of the milk produced by the three different breeds.

For each grazing system, the carotene data for the milk from the three breeds and for the pasture herbage were grouped chronologically by semi-monthly periods for each grazing season. Subsequently these data for the three seasons were averaged by the corresponding calendar periods. This organization of the data was designed to show trends ascribable to seasonal changes as well as to indicate variations resulting from the different systems of grazing.

RESULTS

The results as shown in the graphical summary of the data, figure 1, are essentially negative; that is, the system of grazing effected no marked and consistent differences in the carotene of either the pasture plants or the milk.

In the case of the carotene concentration of the herbage, the only notable difference that could be related to the system of grazing occurred in June when there was a transition from leguminous plants to Bermuda grass. Since the carotene of plants generally bears a direct relationship to their rate of growth, the observed difference at this period might be ascribed to the fact that the continual grazing retarded the rate of recovery of the pasture herbage to a greater degree than did the rotational grazing.

Irrespective of the system of grazing, the trends and fluctuations in the carotene of the herbage reflected the influences of several interrelated modifying factors, most pronounced of which were stage of maturity and rainfall. When the clovers and vetches were flourishing in the spring and Bermuda grass was in its initial stages of growth in the early summer, the carotene concentrations were higher than at any other time. The effect of maturation, accompanied by droughts in late spring and early fall, was indi-

cated by the interrupted decreases in the Bermuda grass, first, following the initial flush in June and, second, after the marked growth retardation in early fall.

A comparison of the carotene values of the milk produced from the respective systems of grazing disclosed no pronounced differences that might be ascribed to the systems of grazing. This observation is in accord with expectations since the yield (18) and the carotene values of the herbage from the comparative systems were similar.

The trends of the carotene of the milk, for the most part, were typical for normal pasture grazing. The precipitous rise during the first two weeks following the change of the feeding regime from winter roughage to pasture herbage is in agreement with long-established precedent (14), but the subse-

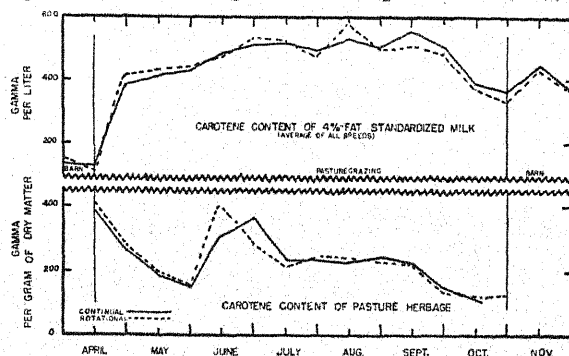


FIG. 1. Summary of a three-year pasture investigation showing the comparative effects of continual and of rotational grazing on the carotene concentration of the pasture herbage and of the milk produced therefrom.

quent retarded rate of increase to the maximum plateau, apparently the "ceiling" value (16), is somewhat unusual (11). Evidently a decrease in the carotene intake resulting from the maturation of the spring legumes was the primary factor contributing to this retardation. A probable secondary operative element was the increased output in the milk; even though the amount of carotene eliminated through this channel constitutes only a small fraction of the total amount ingested (3, 4, 6, 8, 12, 15). The changes in the milk yield during May and June were incommensurate with the reduction in the availability of fresh green herbage. Therefore, the additive results of these relative changes in intake and output would account, in a great measure, for the prolongation of the time necessary to reach equilibrium.

The temporary interruption of the decline of carotene concentration of the milk coinciding with the change from grass to silage might be attributed primarily to an increased intake of carotene. The fresh silage was more palatable and probably more potent in carotene than the Bermuda grass in an advanced stage of maturity.

DISCUSSION

The emphasis placed on the rôle of carotene as the primary source of vitamin A active substances in dairy cattle nutrition has led to the exploration of a multitude of factors affecting the concentration of this compound in the plants commonly used for dairy feeds. Grazing *per se* probably affects the carotene of permanent pasture herbage to the extent that it modifies the direct influence of other factors. Grazing practices that either impair plant development or permit the plants to reach an advanced stage of maturity obviously adversely affect carotene values.

In the foregoing comparison of continual and rotational systems of management the intensity of grazing was regulated so that optimum returns were obtained from lactating cows throughout the grazing season. Since the carrying capacity of the pastures under the two systems of grazing was approximately the same, the similarity in carotene values was in accord with expectations when these observations are considered in the light of the vital rôle that the quality of forage plays in milk production.

It is reasonable to assume that any pasture providing the quality and the quantity of herbage necessary to maintain optimum milk yields over a prolonged period also would supply carotene in sufficient amounts to raise its concentration in the milk to the "ceiling" value. Irrespective of system of grazing the uniform level of carotene in the milk (figure 1) during the summer months and the lag in its reduction in relation to the initial decrease of carotene in the herbage, during the latter part of summer, tend to support the foregoing assumption. However, a possibility that cannot be disregarded entirely is that the intake-output relationship of the carotene during the summer months might have been so constant that a uniform level in the carotene of the milk was maintained at a "sub-ceiling" level.

During the latter part of the grazing season, the decrease in the carotene of the milk following that of the herbage indicates that either the cows did not have a carotene reserve or that the reserve was not being utilized in sufficient amounts to maintain the prevailing high level in the milk. This last suggestion appears to be the more rational explanation in view of the present knowledge of depletion of body stores.

Even though the observations of this investigation disclosed no significant differences that might be ascribed to the system of grazing, the results do not preclude the possibility that the two systems may reveal a different relationship, particularly in the carotene of the herbage, with other species of plants and/or different soils and fertilizers.

SUMMARY

A study was made of the relation of continual and rotational systems of grazing to the carotene content of the herbage of improved Bermuda grass

pastures and of the milk produced by respective groups of cows grazing thereon.

The system of grazing effected no marked and consistent difference in either the concentrations or the variations of the carotene in the herbage.

The carotene concentrations and fluctuations of the milk revealed no differences that could be ascribed to the system of grazing.

The quantitative changes in the carotene ingested were reflected in the carotene values of the milk during the spring and the fall when the concentrations in the milk were at a "sub-ceiling" level, but this did not hold true during the summer, since the values of the milk apparently were at "ceiling" level.

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OXIDIZED FLAVOR IN MILK. XIII. STUDIES OF CUPRIC COM-
PLEXES OF ASCORBIC ACID AND CERTAIN AMINO ACIDS
AND THEIR POSSIBLE RELATIONSHIP TO OXIDIZED
FLAVOR DEVELOPMENT IN MILK*

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In studies on the mechanism for the production of oxidized flavor in milk Olson and Brown (4) postulated that the ascorbic acid in milk was oxidized by copper ions giving hydrogen peroxide as a product; the hydrogen peroxide was then thought to oxidize the phospholipides of milk, producing the oxidized flavor. It was found, however, that excess ascorbic acid prevented the formation of oxidized flavor. This would seem to be an anomaly. However, this might be explained if ascorbic acid formed a complex ion with copper.

Wark (7), in studying the complex ions of hydroxy acids with copper, stated: "The capability of any hydroxy-compound to form complexes with metallic oxides must depend on the acidity of the hydroxyl group. The more strongly this group functions as an acid, the more stable will the complexes be." From this it would seem that the highly acidic hydroxy group of ascorbic acid could form a complex.

Morton (3) reported that in neutralized solutions copper complexes were formed with tartaric, citric, malic, and salicylic acids. In alkaline solution these complexes were destroyed with the formation of highly basic hydrosols. In contradiction to Wark (7) Morton further stated that no stable copper complexes were formed with glyceric, lactic, glycollic, or mandelic acids, the apparent stability of the cupric salts of these hydroxy acids in the presence of caustic alkalies being due to the peptizing properties of the hydroxy-acid anion.

Any compound that forms a copper complex would help prevent the oxidation of the normal ascorbic acid in milk and thus inhibit the formation of oxidized flavor. Sherwood and Hammer (5) have reported concentrations of citric acid salts ranging from 0.07 per cent to 0.33 per cent in milk. In addition, the proteins of milk should form copper complexes although there is some dispute as to the type of complex formed.

Proteins may form complexes with copper through the free amino groups. Thus they would act similarly to amino acids. Borsook and Thimann (1)

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studied the equilibrium relations existing in solution between copper ions and glycine and alanine. They were studied by measurement of absorption spectrum and by copper electrode potentials through a range of hydrogen ion concentrations from pH 0 to 13. They found four types of complexes of both glycine and alanine, depending on the pH of solution.

Smythe and Schmidt (6) studied the formation of complexes of sixty-one compounds with iron. This would be similar to the complexes of copper. By calculating the residual negative charge of the nitrogen atom in amino acids, they reasoned that monoamino monocarboxylic acids would not form a complex. Their experimental work confirmed this. However, they found that dicarboxylic acids and hydroxy acids did form complexes. In addition they claimed the formation of a complex with phosphoric acid and iron due to the residual negative charges on the hydroxy groups of phosphoric acid. The formation of an iron complex with casein was claimed to be due to the phosphoric acid in casein. These studies were done at pH 2.5.

The purpose of the present study was to determine whether or not copper complexes were formed with ascorbic acid and amino acids. Citric acid was also used for comparative purposes. β -alanine was used to compare the action of α -amino and β -amino acids.

EXPERIMENTAL

Most determinations of copper complexes are done with relatively high concentrations of copper. In this work it was desired to work in the range of copper concentration that might be found in milk, that is, about one part per million. For this purpose a method for determining very small amounts of ionized copper was sought. A modified Biazzo (2) method was found applicable. By using a 10-centimeter tube in a Kuffel and Esser color analyzer at a wave length of 450 m μ ., a range of copper concentration from 0.01 mg. to 0.1 mg. was accurately determined. The copper content of the water and reagents was estimated by using a water blank with the color reagents against the different copper concentrations. By plotting $-\log T$ against concentration of copper a straight line parallel to the line obtained by running the various copper concentrations against a chloroform blank was obtained. From the distance between the lines the copper content of the reagents and water was found to be from 0.01 mg. to 0.015 mg. for the various batches of redistilled water used.

Although it was desirable to determine the amount of copper ionized at pH 6.5 to 7.0, this method (2) used acetic acid for the development of color and, consequently, the amount of copper ionized was actually determined at a pH of about 3.5. According to Morton (3) the complex of copper should be determined at neutrality. Ascorbic acid and citric acid with copper showed a diminution of copper ions, but these values would have to be considered minimum values. The amino acids showed very little diminution

of copper ions at the pH of reaction. Since Borsook and Thimann (1) have shown that a different complex of amino acids is formed at pH 3.5 than at pH 6.5, this method was not used for the determination of the amino acid complexes.

The amount of ionizable copper with citric acid was determined by adding concentrations of 0.1 per cent to 1.0 per cent of citric acid neutralized to pH 6.5, to 0.1 mg. of copper and adding the reagents to develop color to a total volume of 100 ml. The color was extracted from the water solution with 25 ml. of chloroform, the chloroform was centrifuged for a few min-

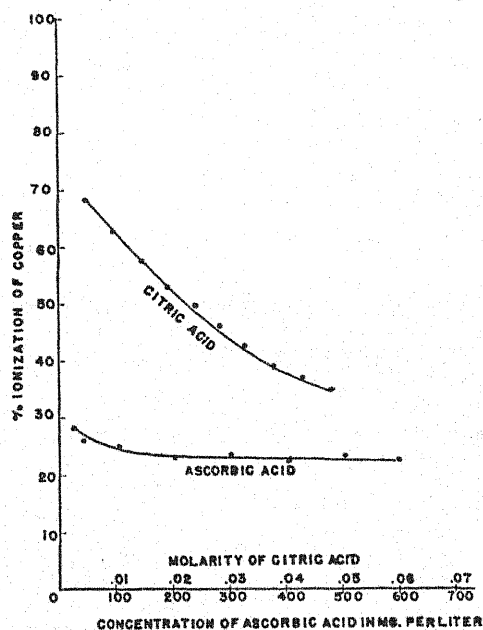


Fig. 1. Ionization of copper with citric and ascorbic acids.

utes to separate water droplets, and the color was immediately read in the color analyzer.

In a similar way the ionization of copper was determined with ascorbic acid, using concentrations of 20 mg. to 600 mg. per liter.

The results of both the citric acid and the ascorbic acid are shown in figure 1. They are calculated as the percentage of the total copper ionized. The curve for citric acid shows results typical for a complex, that is, the higher the concentration of citric acid the less copper was ionized. The ascorbic acid shows an entirely different type of curve. The amount of ionizable copper remains practically the same for all the concentrations of ascorbic acid. Instead of a typical complex like that of citric acid, the ascorbic acid seems to form a definite compound of copper ascorbate. The ascorbic acid ties up all but about 25 per cent of the copper very definitely.

It was desired to see what effect the copper complex of citric acid had on the protection of ascorbic acid. In a preliminary trial it was found that copper in a concentration of one part per million in 13 minutes completely destroyed, 40 mg. per liter of ascorbic acid in a 0.05 m. phosphate buffer of pH 6.8. This 13-minute period was therefore used as a comparative time interval. A series of 100-ml. volumetric flasks containing 0.1 mg. copper, the phosphate buffer at pH 6.8 and neutralized citric acid in concentrations of 0.1 per cent to 1.0 per cent was set up. Ascorbic acid to make a concentration of 40 mg. per liter was added, the flask filled to volume quickly,

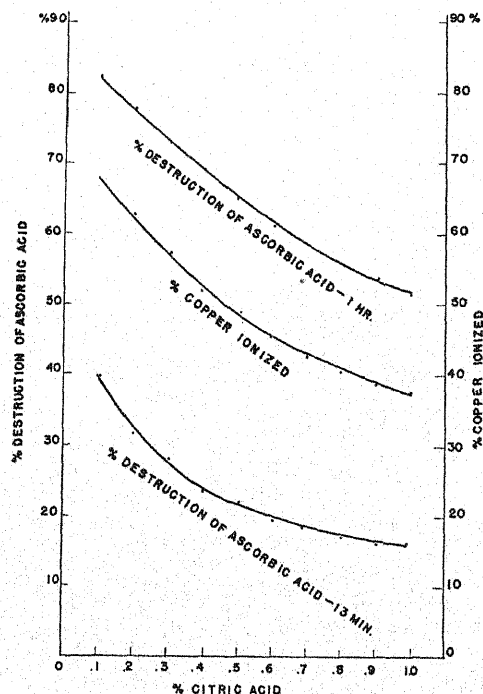


FIG. 2. Ionization of copper and destruction of ascorbic acid (40 mg./l.) with citric acid.

mixed, and placed in the dark. After exactly 13 minutes an aliquot was removed and titrated with a solution of sodium 2,6-dichlorobenzenoneindophenol to determine the amount of ascorbic acid remaining. A similar aliquot was titrated after one hour.

The results are shown in figure 2. For comparison the curve for ionizable copper with citric acid is also shown. It is readily seen that less ascorbic acid is destroyed with increasing concentration of citric acid. The citric acid protects the ascorbic acid from destruction. In addition the two curves for the destruction of ascorbic acid are similar in shape to the curve for ionizable copper, particularly the curve for one hour. These curves

show definitely that the inhibition of ascorbic acid destruction is due to the removal of copper ions by the citric acid complex.

The same method was used to determine whether or not amino acids prevent the destruction of ascorbic acid by copper. The experiments were conducted exactly the same as for citric acid, using glycine, glutamic acid, alanine, and β -alanine in the same molar concentrations. Thus results were obtained for two monoamino monocarboxylic amino acids, one monoamino dicarboxylic acid, and one β -amino acid. Citric acid was also run for comparative purposes. The results are the average of two or three determinations.

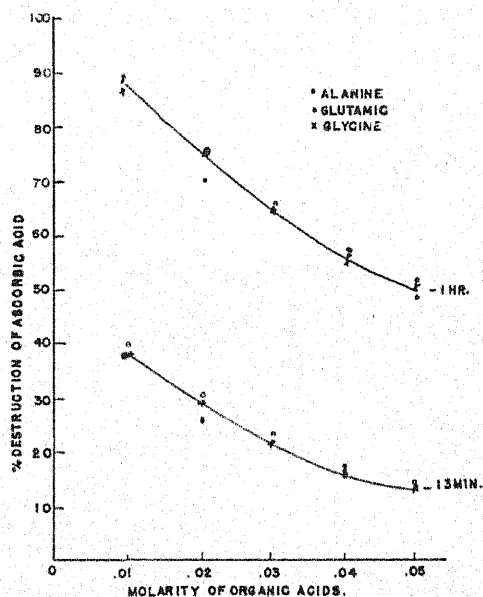


FIG. 3. Protection of ascorbic acid by complex ions of α -amino acids.

The results of the amino acids as inhibitors of the destruction of ascorbic acid are shown in figures 3 and 4. It will be noted in figure 3 that glycine, glutamic acid, and alanine all fall along the same curves. The variation from the curves is no more than the experimental error in the individual determinations. It may be noted in figure 4 that citric acid forms a curve of different shape from the amino acids (glycine, glutamic, and alanine) but of close to the same protective value. Glutamic acid gives the same curve as glycine and alanine, contrary to Smythe and Schmidt's (6) hypothesis that the two carboxyl groups form the complex and not the amino group. Furthermore it must be remembered that the amino acids are compared to the destruction in phosphate buffer which completely destroys the ascorbic acid in thirteen minutes. Smythe and Schmidt's (6) contention that phosphoric acid forms a copper complex is not supported by these results.

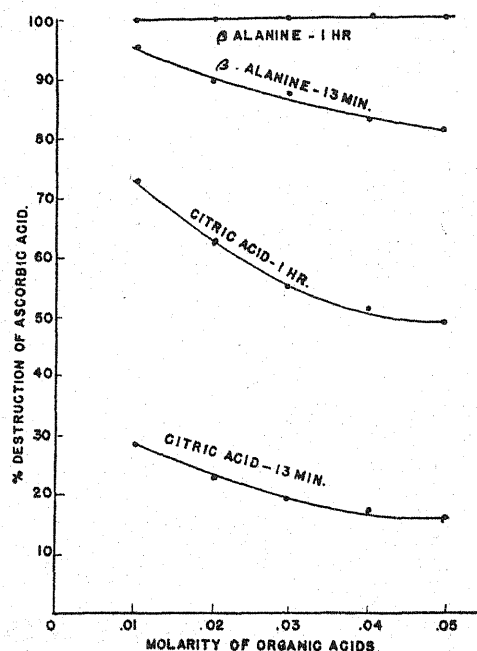


FIG. 4. Protection of ascorbic acid by complex ions of citric acid and β -alanine.

Finally the curves in figure 4 for β -alanine show that the amino group in the β position forms a very weak complex. After one hour the ascorbic acid is totally destroyed regardless of the concentration of β -alanine. These results would definitely indicate that amino acids form copper complexes through the amino group.

TABLE 1

The effect of varying amounts of citric acid on the development of oxidized flavor in buffered washed cream containing 42 mg. per liter of ascorbic acid and 1.0 ppm. copper

	Date					
	8/19/42	8/20/42	8/21/42	1/9/43	1/12/43	1/15/43
Control	OX	OX	OX	OX	OX	OX
Washed cream						
plus citric acid						
0.05%	OX
0.1%	OX	OX	OX
0.2%	?	sl. ox	sl. ox	OX	ox	sl. ox
0.3%	?
0.4%	—
0.5%	—*	?	—	—*	sl. ox	—
0.6%
0.7%	—*	—*
0.8%
0.9%	—*	—*
1.0%	—*	—*	—*	—

Meaning of symbols: * bitter from citrate; ox oxidized flavor; — no oxidized flavor.

TABLE 2

The effect of citric acid on the development of oxidized flavor in susceptible milk contaminated with 1.0 ppm. copper

	Date				
	1/8/43	1/14/43	1/18/43	1/19/43	1/20/43
Control	ox*	ox	ox	ox	ox
Milk plus citric acid 0.02 M.	—	—	—	—	—

* For meaning of symbols, see table 1, footnote.

Since citric acid has been shown to have a protective action against the oxidation of ascorbic acid by the copper ion it seemed desirable to determine its effect on the development of oxidized flavor in washed cream and milk. Accordingly, samples of washed cream were prepared and tested according to the method of Olson and Brown (4). The results of these experiments

TABLE 3

The effect of adding alanine and glycine on the susceptibility of washed cream containing 42 mg. per liter of ascorbic acid and 1.0 ppm. copper to the development of oxidized flavor

	Date				
	12/31/42	1/9/43	1/12/43	1/15/43	1/19/43
Control	ox*	ox	ox	ox	ox
Conc. of alanine					
0.02 M.	ox	ox	sl. ox	sl. ox	ox
0.05 M.	sl. ox	sl. ox	sl. ox	—	sl. ox
0.10 M.	—	?	—	—	v. sl. ox
Conc. of glycine					
0.02 M.	ox	ox	ox	sl. ox	sl. ox
0.05 M.	sl. ox	sl. ox	sl. ox	sl. ox	?
0.10 M.	—	?	v. sl. ox	—	—

* For meaning of symbols, see table 1, footnote.

are shown in tables 1 and 2. These results confirm the finding that citric acid has an inhibitory effect on the development of oxidized flavor by ascorbic acid.

Likewise, the effect of alanine and glycine was studied on both washed cream and milk. These results are shown in tables 3 and 4.

TABLE 4

The effect of adding alanine and glycine on the susceptibility of milk containing 1.0 ppm. of added copper to the development of oxidized flavor

	Date				
	1/8/43	1/14/43	1/18/43	1/19/43	1/20/43
Control	ox*	ox	ox	ox	ox
Conc. of alanine					
0.02 M.	—	—	—	—	—
0.05 M.	—	—	—	—	—
Conc. of glycine					
0.02 M.	—	—	—	—	—
0.05 M.	—	—	—	—	—

* For meaning of symbols, see table 1, footnote.

Here, likewise, both alanine and glycine are found to have an inhibitory action on the development of oxidized flavor. This inhibitory action was not as strong as in the case of the citric acid.

DISCUSSION OF RESULTS

These results indicate that the development of oxidized flavor in milk is closely associated with the ionization of copper and its destruction of ascorbic acid. Apparently anything which decreases the ionization of copper will in turn retard the destruction of ascorbic acid and in this manner tend to retard oxidized flavor development. As a result of these findings, it seems that many individual factors may have a bearing on the susceptibility or nonsusceptibility of milk to the development of oxidized flavor.

CONCLUSIONS

1. Ascorbic acid forms either a complex or a direct compound with copper ions.
2. The protective action of citric acid on the oxidizing action of copper ions with ascorbic acid is due to the formation of a copper complex with citric acid thus removing copper ions from the reaction.
3. Through the indirect evidence of inhibition of ascorbic acid destruction with copper ions, it is shown that the amino acids—glycine, glutamic acid, and alanine—are equally effective in forming complex ions. With the evidence that β -alanine forms a very weak complex, this shows that the amino acids form copper complexes through the free amino group.

ACKNOWLEDGMENT

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OXIDIZED FLAVOR IN MILK. XIV. A POSSIBLE MODE OF ACTION OF INHIBITORS IN PREVENTING THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK*

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Various substances have been found to inhibit the formation of oxidized flavor in milk. Dahle and Palmer (5) found that excess ascorbic acid protects against oxidized flavor. Anderson (1) reported that a pancreatic enzyme added to milk just before pasteurization protects the milk against oxidized flavor. Corbett and Tracy (4) found that both tyrosine and the butyl ester of tyrosine prevented the flavor, and that "Enzylac" and Avenex gave protection. Even ordinary raw sugar gave some protection. Williams and Burgwald (16) found that carotene did not protect, but that mixed tocopherols gave a protection. Garrett (7) found that divalent manganese protected milk against oxidized flavor. Archibald (2) fed an ounce of manganese sulphate daily to cows and analyzed the milk. The manganese content of the milk was doubled, but even this amount was not sufficient manganese to prevent oxidized flavor. Brown and Olson (3) found that potassium iodide added directly to the milk protected against oxidized flavor.

During the summer of 1942 the authors attempted to isolate the active inhibitor in summer milk. The casein was precipitated at the isoelectric point. This was made into calcium caseinate by the method of Palmer (13). The calcium caseinate, whey, and sterilized whey, were then tested on washed cream according to the method of Olson and Brown (12). In most cases the oxidized flavor was prevented. In addition lactose was tried and found to inhibit oxidized flavor. Thus it was found that a number of naturally occurring substances in milk inhibit oxidized flavor.

Brown and Olson (3) proposed a chemical theory for the development of oxidized flavor in milk. This theory postulated that cupric copper oxidizes the ascorbic acid in milk to dehydroascorbic acid with the production of copper in the cuprous state of ionization. The cuprous copper is then oxidized back to the cupric form by dissolved oxygen with the production of hydrogen peroxide. The hydrogen peroxide in turn oxidizes the phospholipides in milk to give the oxidized flavor.

The purpose of this paper was to study the natural inhibitors in milk as well as the inhibitors proposed by other workers in order to test this theory

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of oxidized flavor development. To test this the inhibitors were tried again on milk and wherever possible on washed cream to be sure that a preventive for oxidized flavor was actually present. A chemical explanation was then attempted for the inhibiting action.

Referring again to the theory of the development of oxidized flavor, it is readily seen that it can be prevented (I) by removal of copper ions so that the ascorbic acid is not oxidized. A class of inhibitors in this group are those that form complex ions with copper thus reducing the copper ions in solution. A second class (II) would include substances that are themselves oxidized by hydrogen peroxide before the hydrogen peroxide oxidizes the phospholipides of milk. Because of a lack of knowledge of the chemistry involved, a third class (III) must be added to include substances that inhibit oxidized flavor but for which the mechanism is not understood.

EXPERIMENTAL RESULTS

I. The first class of inhibiting substances in which the ionization of copper is repressed is typical of salts of citric acid and amino acids as cited by Olson and Brown (11). They found that hydroxy organic acids form a complex with copper which inhibits the ionization. This in turn inhibits the oxidation of ascorbic acid and consequently the oxidized flavor formation. The effect of amino acids was tried in order to gain an insight into the action of proteins. From the results with amino acids it is seen that the inhibiting effect of proteins would be caused by the free amino and carboxyl groups of the proteins.

Casein. Calcium caseinate, made according to the method of Palmer (13), was tried as an inhibitor on washed cream according to the method of Olson and Brown (12). The results are shown in table 1. These results show that calcium caseinate in a three per cent solution protects washed cream against oxidized flavor. In two cases oxidized flavor was inhibited by a percentage lower than the casein content of milk. This would indicate that in the preparation of the calcium caseinate, it was partly hydrolyzed and the liberated free amino groups inhibited the oxidized flavor.

Proteolytic enzymes. Since "Enzylac" includes a proteolytic enzyme, the inhibiting effect of this product may be due to a partial hydrolysis of milk proteins which liberate free amino groups to form complex ions with copper. That "Enzylac" does protect milk against oxidized flavor is shown in table 2 where the enzyme was added according to the directions of the manufacturer.

Ascorbic acid. Another substance belonging to this class is ascorbic acid, as shown by the results of Olson and Brown (11). The presence of excess ascorbic acid prevents the ionization of copper probably through the formation of a copper compound. In this manner the ascorbic acid differs from the amino acids which form complexes as indicated by decreased ionization of copper with increased concentration of the amino acids.

TABLE 1

The effect of various concentrations of calcium caseinate on the susceptibility of washed cream containing 42 mg./liter of ascorbic acid to oxidized flavor

Date	Control Washed cream ppm. Cu				3% calcium caseinate plus ppm. Cu				2% calcium caseinate plus 1.0 ppm. Cu	1% calcium caseinate plus 1.0 ppm. Cu
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5		
7/30/42	ox*	ox	ox	ox	—	—	—	—
8/12/42	sl. ox	ox	ox	ox	—	—	sl. ox	ox
8/14/42	ox	ox	ox	ox	—	—	—	—
12/30/42	ox	ox	ox	ox	sl. ox	sl. ox	sl. ox
1/15/43	ox	ox	ox	ox	—	—	—
1/19/43	ox	ox	ox	ox	—	—	—

* Meaning of symbols: ox, oxidized flavor; —, no oxidized flavor.

II. The second class of substances includes those which inhibit oxidized flavor by the substances themselves being oxidized by hydrogen peroxide such that the phospholipides of milk are not oxidized. Lactose, avenex, potassium iodide, and manganese showed very little or no protection of ascorbic acid against oxidation when tried according to the method used by Olson and Brown (11) and therefore do not form complexes with copper. Tyrosine showed a very slight effect in 0.5 per cent suspension but no more than could be attributed to the small amount of amino acid present in solution.

Tyrosine. The effect of tyrosine as an inhibitor of oxidized flavor both with washed cream and milk is shown in table 3. The results show that 0.1 gram of tyrosine per half pint inhibits oxidized flavor in both milk and washed cream although it may not entirely eliminate the flavor. Since tyrosine is quite insoluble, no higher concentration could be tried. The action of tyrosine as an inhibitor is explained by Mystkowski (10). He has shown that tyrosine added to a solution of ascorbic acid and copper inhibits oxygen evolution and the tyrosine changes to a reddish brown color corresponding to quinone products of tyrosine oxidation. Thus it is seen that tyrosine inhibits oxidized flavor in milk by removing the hydrogen peroxide

TABLE 2

The effect of "Enzylac" as an inhibitor of oxidized flavor on susceptible milk

Date	Control milk				Milk + "Enzylac"			
	ppm. Cu				ppm. Cu			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/11/43	—	ox	ox	ox	—	—	—	—
1/14/43	—	ox	ox	ox	—	—	—	—
1/19/43	—	ox	ox	ox	—	—	—	—
1/20/43	—	—	ox	ox	—	—	—	—

For meaning of symbols, see table 1, footnote.

formed in the ascorbic acid oxidation. Avenex, being a natural product mixture, might contain some quinone type compound which could prevent oxidized flavor similar to tyrosine.

Manganese. It would seem to be very strange that divalent manganese would be an inhibitor of oxidized flavor. However, the results in table 4 show that manganese in sufficient quantities definitely prevents oxidized flavor in milk. The following explanation seems possible. When sodium hydroxide is added to a divalent manganese salt a light tan precipitate immediately forms. On exposure to air for a few minutes the top of the precipitate turns black with the formation of $MnO(OH)_2$ due to oxidation by atmospheric oxygen. Similarly if a manganese salt is added to a citrate buffer at a pH of 6.5-7.0, a very light-colored solution results. On standing

TABLE 3

The effect of the addition of 0.1 gram of tyrosine per half-pint on the development of oxidized flavor in milk and washed cream

Date	Milk								Washed cream (buffered)		Tyrosine
	Control				Milk + 0.1 gr. tyrosine				Control	Washed cream+tyrosine	
	ppm. Cu				ppm. Cu				1.0 ppm. Cu	Plus 42 mg./l. ascorbic acid 1.0 ppm. Cu	
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5			
12/31/42	—*	—	—	—	OX	OX	0.01 M
1/ 8/43	—	OX	OX	OX	—	—	—	—
1/12/43	OX	OX	0.1 gr.
1/14/43	—	OX	OX	OX	—	—	—	sl. OX
1/15/43	OX	—	0.1 gr.
1/18/43	—	—	OX	OX	—	—	—	—
1/19/43	—	—	OX	OX	—	—	—	—
1/20/43	—	—	OX	OX	—	—	—	—

* For meaning of symbols, see table 1, footnote.

in the air this gradually becomes a dark brown, indicating oxidation by atmospheric oxygen. Thus manganese either protects milk from oxidized flavor by absorbing the hydrogen peroxide in the ascorbic acid oxidation process, or it may even absorb the dissolved oxygen in the milk thus preventing the oxidation of the copper to form hydrogen peroxide.

Sugars. Lactose shows some inhibition of oxidized flavor as is seen in table 5. Since lactose does not form a complex ion with copper, the only possible explanation for this effect is that the potentially free aldehyde group in lactose is oxidized by hydrogen peroxide. To test this further, sucrose was tried on washed cream. Sucrose does not have a potential aldehyde group and it did not inhibit oxidized flavor. Glucose on the other hand is a simple sugar with a potential aldehyde group. As shown in table 6, glucose did tend to prevent oxidized flavor. That glucose is capable of being oxidized by hydrogen peroxide was shown by Witzemann (17) who found that glucose could be completely oxidized to carbon dioxide and water by

TABLE 4

The effect of various concentrations of manganese on the susceptibility to oxidized flavor of milk and washed cream contaminated with copper

Date	Milk									
	Control + Cu				Control + 1.5 ppm. Cu + Mn					
	ppm. Cu				ppm. Mn					
	0.0	0.5	1.0	1.5	0	1	2	3	4	5
1/ 8/43	—*	ox	ox	ox	ox	—	—	—
1/14/43	—	ox	ox	ox	ox	ox	—	—
1/18/43	—	sl. ox	sl. ox	ox	ox	—	—	—
1/19/43	—	—	ox	ox	ox	ox	—	—
1/20/43	—	—	ox	ox	ox	—	—	—
Washed cream (buffered) containing added ascorbic acid, 42 mg./l.										
					Control + 1.0 ppm. Cu + Mn					
					ppm. Mn					
					0	1	2	3	4	5
1/ 8/43	ox	ox	ox	sl. ox
1/12/43	ox	ox	ox	sl. ox	v. sl. ox
1/15/43	ox	ox	sl. ox	—	—	—

* For meaning of symbols, see table 1, footnote.

hydrogen peroxide in the presence of disodium phosphate in a pH range of 5.9 to 7.35. If this is the case, it would seem to be easy for the small amount

TABLE 5

The effect of added lactose on the susceptibility of oxidized flavor in milk and washed cream

Date	Control milk				Milk + 2½% lactose				ppm. Cu			
	ppm. Cu				ppm. Cu				Milk + 5% lactose			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/ 8/43	—*	ox	ox	ox	—	—	—	—
1/14/43	—	ox	ox	ox	—	—	—	—
1/18/43	—	—	ox	ox	—	—	—	—
1/19/43	—	—	ox	ox	—	—	sl. ox	sl. ox
1/20/43	—	—	ox	ox	—	—	—	sl. ox
	Washed cream + 42 mg./l. ascorbic acid				Washed cream + 32 mg./l. ascorbic acid + 2½% lactose				Washed cream + 42 mg./l. ascorbic acid + 5% lactose			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
8/20/42	ox	ox	ox	ox	—	—	—	—	—	—	—	—
8/21/42	ox	ox	ox	ox	—	—	—	—	—	—	—	—
1/ 9/43	ox	ox	ox	ox	ox	ox
1/12/43	ox	ox	ox	ox	sl. ox	sl. ox
1/15/43	ox	ox	ox	ox	ox	—
1/19/43	ox	ox	ox	ox	sl. ox	v. sl. ox

* For meaning of symbols, see table 1, footnote.

of hydrogen peroxide formed in the oxidation of ascorbic acid in milk to be used to oxidize glucose or lactose and thus protect from oxidized flavor. That the lactose in milk does not entirely prevent oxidized flavor would be due to both oxidation processes going on at the same time.

The protection from oxidized flavor by tocopherols as found by Williams and Burgwald (16) would be included in this second class of inhibitors also. The tocopherols have a hydroquinone type of structure and are very easily oxidized. The problem as an antioxidant for milk would be to get the tocopherols, which are oil soluble, into a sufficiently fine emulsion to give enough surface for antioxidant properties.

TABLE 6

The effect of glucose on the susceptibility of milk and washed cream to oxidized flavor

Date	Control milk				Milk + 0.5% Glucose				Milk + 1.0% Glucose				Milk + 2.0% Glucose			
	ppm. Cu				ppm. Cu				ppm. Cu				ppm. Cu			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
1/18/43	—*	—	ox	ox	—	—	—	—	—	—	—	—	—	—	—	—
1/19/43	—	—	ox	ox	—	—	—	ox	—	—	—	ox	—	—	—	?
1/20/43	—	—	ox	ox	—	—	—	?	—	—	—	—	—	—	—	—
	Washed cream + 42 mg./l. asc. acid No glucose				Washed cream + 42 mg./l. asc. acid 2½% glucose				Washed cream + 42 mg./l. asc. acid 5% glucose							
	ppm. Cu				1.0 ppm. Cu				1.0 ppm. Cu							
	0.0 0.5 1.0 1.5															
	1/19/43 ox ox ox ox				sl. ox				sl. ox							

* For meaning of symbols, see table 1, footnote.

In connection with this second class of inhibitors, there is also a possibility that an inhibitor may directly oxidize ascorbic acid and thus eliminate the oxidation step by copper. In this way hydrogen peroxide would not be formed and no oxidized flavor in milk would develop. Heard and Welch (8) have found such a system in effect in the adrenal glands. They found that the ascorbic acid directly reduced oxidized adrenaline, forming reduced adrenaline and dehydro-ascorbic acid.

III. A miscellaneous class of inhibitors, which includes those that do not fit well into either group one or group two, should also be considered. In this group are the halides. Brown and Olson (3) found that potassium iodide inhibited oxidized flavor to some extent. Mapson (9) found that the halides—iodides, bromides, and chlorides—showed a protective action on ascorbic acid at pH 2.5 but very little at pH 6 to 7. Mystkowski (10) found good inhibition by sodium chloride in concentrations of 4 per cent to 10 per

cent even at pH 7. This may explain why salted cream used for butter does not develop oxidized flavor. The mechanism of this protective action of the halides is not known. Mapson (9) has postulated a complex ion of the halide with copper.* However, the authors were unable to show any diminishing of the ionization of copper in a solution of potassium iodide, using the method of Olson and Brown (11).

Substances that prevent oxidized flavor by themselves being oxidized by the hydrogen peroxide are of interest. These substances in order to be effective must have an oxidation-reduction potential such that they will be oxidized before the phospholipides. Tyrosine has such an action, but (tyrosine) is not sufficiently soluble in milk to completely prevent the development of oxidized flavor. Divalent manganese, as was found by Garrett (7), inhibited oxidized flavor. An explanation of this is the oxidation of manganous ions to manganic ions by the hydrogen peroxide. That lactose could inhibit oxidized flavor would seem unlikely but the oxidation of the aldehyde group of the sugar and even further oxidation has been shown by Witzemann (17) with glucose.

Since the proteins, citrates, and lactose in milk all have an inhibiting effect on oxidized flavor, it is natural to expect that an increase in any or all of these substances would show an inhibiting effect on winter milk. The work of Russell and Dahle (15) in which added milk solids in the form of condensed milk or dried milk inhibited oxidized flavor development, may be explained on this basis. However, since the condensed milk and dried skim milk may have been subjected to a sufficiently high temperature to liberate sulfhydryl groups, these also might explain the inhibiting action.

DISCUSSION

The results show that a number of substances normally occurring in milk act as inhibitors. These substances—proteins, citrates, and lactose—are present in about the same amount in both summer and winter milk. They show a definite inhibiting action on washed cream. The proteins, particularly, as well as the citrates, to a smaller extent, inhibit by forming complexes with copper, thus removing the copper ions from the reaction. These complexes are formed by the free amino and carboxyl groups of the proteins similar to amino acids. Peterson and Walton (14) have found that various amino acids as well as uric acid inhibit the oxidation of ascorbic acid with copper by forming ions with copper. That it was a complex of copper was shown by the fact that the normal autoxidation of ascorbic acid was not inhibited by the amino acids in the absence of copper. They found that 10^{-4} M cystine was the best inhibitor. Diehl (6) has included a theoretical discussion of copper complexes of amino acids and hydroxy acids through the coordinating groups so as to form chelate rings. These complexes prevented the ionization of copper.

Since proteins inhibit oxidized flavor by the formation of complexes with copper through the free amino and carboxyl groups, any proteolytic enzyme that splits the protein to give more free reactive groups would show a further inhibiting action. This is true of "Enzylac." That the inhibiting effect is not due to the preparation itself is shown by the fact that it does not inhibit oxidized flavor in washed cream.

The search for a special inhibitor present in milk from cows on pasture is complicated by the fact that the normal constituents of milk have an inhibitory action. Such a special inhibitor is entirely possible. However, the results showing that citrates, proteins, and lactose have an inhibitory effect on milk might indicate that the tendency to form oxidized flavor is balanced by these natural inhibitors. Although Brown and Olson (3) found that washed cream containing ascorbic acid and contaminated with copper gives oxidized flavor on pasture milk, the amount of oxidized flavor seemed to be less than that produced on winter milk. This suggests that the phospholipides in winter milk may be more unsaturated than in summer milk. One of the authors has started work on this consideration.

SUMMARY

A possible explanation for various inhibitors for oxidized flavor is presented. One class of substances which includes proteins, citrates, and amino acids inhibits by forming complex ions with copper, thus repressing its ionization.

Tyrosine, divalent manganese, lactose, and glucose inhibit oxidized flavor by being oxidized by hydrogen peroxide, thus protecting the phospholipides of milk.

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THE EFFECT OF MACHINE MILKING UPON THE LEUCOCYTE COUNT AND THE CHLORIDE CONTENT OF MILK

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INTRODUCTION

In an earlier paper (2) it was shown that the leucocyte count and the chloride content of milk are not reliable indices of udder infection. Individual differences between cows with respect to these two tests and differences in results caused by factors other than infection frequently are greater than the changes that occur when the udder becomes infected. Any factor that causes udder irritation doubtless will cause a rise in the leucocyte and chloride values.

The extent to which machine milking may influence leucocyte counts and chloride determinations, or other mastitis tests that reflect udder irritation, such as pH and catalase tests, is not clear. Munch-Petersen (4) reviewed the literature on mastitis and found opinion about equally divided with respect to milking machines as a factor contributing to mastitis. Dahlberg (1) found that leaving machines on the cows too long contributed to udder troubles and resulted in a drop in production early in the lactation period. He presented data indicating that reducing to 4 or 5 minutes the time interval that the machines were left on the cows was accompanied by a drop in the leucocyte count of the mixed herd milk. Hucker (3) reported that the leucocyte count of milk from machine-milked cows was higher than that of milk from hand-milked cows. In his machine-milked group there was a higher incidence of streptococcic infections than in his hand-milked group, a factor which probably exaggerated the difference he found between the two groups with respect to leucocyte counts.

In studies on the effects of milking machines on tests for mastitis there has not to the author's knowledge been any report which deals separately with infected and uninfected cows. If milking machines cause irritation which predisposes the udder to infection, one should expect detectable increases in the leucocyte count and chloride content of the milk when a cow is on machine milking as compared with values found for the same cow when she is on hand milking. On the other hand, if the machine is only a fomite spreading the infection without itself predisposing the udder to the infection, high leucocyte counts and chloride values would be found in the milk of machine-milked cows because of the high incidence of infection and not directly as a result of injury caused by the machines. The purpose of this

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paper is to report a study in which the effects of machine milking on cows are considered separately for infected and uninfected quarters.

METHODS

The data presented in this report were collected during the period from 1936 to 1942 in an investigation on mastitis in the Bureau of Dairy Industry herd at Beltsville, Maryland. The plan of the investigation was to begin with uninfected first calf heifers and continue them on the experiment as long as they remained in the herd. At intervals of 2 to 4 weeks individual quarter samples of the foremilk were taken from each cow and examined in the laboratory.

The examination consisted of the Hotis test, plating on Edwards aesculin crystal violet blood agar, a direct microscopic count of leucocytes and direct titration of chlorides with silver nitrate and a potassium chromate indicator. When there were abnormalities that could not be explained by the results on the Hotis test and by plating on the highly selective Edwards medium, the samples were plated in addition on standard tryptone glucose meat extract milk agar. Representative colonies were picked from the plates into meat infusion broth for identification of the organisms.

The herd at Beltsville is maintained primarily for experiments on feeding, breeding, and management. Changes from hand milking to machine milking or vice versa were made from time to time either in the interests of production records or for convenience in management. Usually when an animal was put on either hand or machine milking at the beginning of the lactation period, she remained on the same method of milking for the entire period. Occasionally, however, a change from one method of milking to the other was made during the lactation period. Under these circumstances it was possible to obtain data on 31 different cows that were on both methods of milking during the course of the investigation. For a few cows the data for a given method of milking extend over only parts of lactation periods, but for most of the cows one or more lactation periods were covered with each method of milking.

The data on the leucocyte counts and the chloride content for the different quarters have been divided into groups not only on the basis of the presence or absence of infection as determined by cultural tests, but also on the basis of the stage of lactation. For this purpose the lactation periods were divided into intervals of three months each. The data were also summarized for the entire lactation period.

THE EFFECT OF THE METHOD OF MILKING ON UNINFECTED QUARTERS

Table 1 shows the results obtained from the samples from the uninfected quarters of 27 cows. Of a total of 2,883 samples considered in this table, 1,571 were obtained while the cows were on hand milking and 1,312 while the cows were on machine milking.

TABLE 1

The effect of machine milking versus hand milking on the leucocyte count and chloride content of milk from uninfected quarters

Cow No.	First 3 months of lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	<i>number</i>	<i>per cent</i>	<i>thousands</i>	<i>number</i>	<i>per cent</i>	<i>thousands</i>
820	18	0.107	29	12	0.119	104
1235	6	0.128	82	15	0.186	804
1247	16	0.108	36
1265	20	0.099	26
1268	24	0.122	54	24	0.138	426
1269	13	0.110	287	35	0.113	269
1273	24	0.127	72	20	0.118	323
1274	20	0.111	31	14	0.102	235
1275	12	0.120	122	27	0.185	892
1278	16	0.124	52	10	0.111	310
1279	20	0.103	44	13	0.101	138
1282	32	0.105	24	24	0.130	488
1294	21	0.120	174
1298	12	0.111	48	28	0.128	119
1418	12	0.114	459
1434	33	0.120	77
1441	4	0.109	122
1445	16	0.094	26	47	0.090	188
1472	10	0.101	34	18	0.096	187
1473	10	0.088	22	32	0.110	917
1474	24	0.106	64	32	0.104	177
1476	16	0.116	159	19	0.104	1004
1478	9	0.097	302
1483	12	0.110	116
1488	15	0.089	23	21	0.099	372
1489	8	0.086	20
1490	14	0.090	229	21	0.094	90
Total or averaget	376	0.110	78	473	0.116	365
3 to 6 months in lactation						
820	15	0.117	20	15	0.132	257
1235	15	0.125	50	9	0.161	1051
1247	3	0.111	103	13	0.107	116
1265	24	0.106	99
1268	40	0.116	94
1269	15	0.102	200	28	0.117	186
1273	20	0.119	91	24	0.132	145
1274	16	0.114	75	28	0.106	170
1275	20	0.130	112	4	0.189	1402
1278	4	0.117	18	22	0.139	103
1279	18	0.102	128
1282	40	0.113	25	16	0.143	605
1294	34	0.119	88	12	0.120	369
1298	24	0.130	106
1418
1434	15	0.122	36	13	0.127	30
1441	24	0.116	339
1445	22	0.105	44	37	0.095	45
1472	10	0.103	65
1473	10	0.091	36	16	0.110	408
1474	20	0.116	306	36	0.119	637
1476	23	0.108	142
1478	22	0.101	151
1483	20	0.102	31	7	0.109	54
1488	16	0.096	23	8	0.091	169
1489	8	0.093	27	2	0.109	36
1490	18	0.101	318
Total or averaget	366	0.113	88	419	0.117	258

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

TABLE 1—(Continued)

Cow No.	6 to 9 months in lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	<i>number</i>	<i>per cent</i>	<i>thousands</i>	<i>number</i>	<i>per cent</i>	<i>thousands</i>
820	12	0.126	54	9	0.186	383
1235	12	0.119	51	2	0.213	1505
1247	3	0.110	85	8	0.125	109
1265	14	0.134	190	4	0.121	45
1268	36	0.133	184
1269	15	0.109	31	9	0.128	51
1273	20	0.137	149	16	0.162	351
1274	32	0.112	60	4	0.127	471
1275	20	0.161	346
1278	16	0.159	385
1279	24	0.106	191
1282	40	0.119	20	4	0.240	2292
1294	6	0.111	76	8	0.136	439
1298	12	0.113	23	16	0.155	477
1418	4	0.097	429
1434	23	0.142	156	26	0.139	116
1441	9	0.106	73	4	0.128	571
1445	14	0.112	92	19	0.124	159
1472	12	0.101	27	4	0.110	95
1473	10	0.105	388	8	0.112	365
1474	20	0.123	208	25	0.141	991
1476	30	0.107	116
1478	20	0.107	148
1483	16	0.107	69	12	0.124	165
1488	22	0.102	44
1489	6	0.102	18	8	0.107	36
1490	6	0.123	239
Total or averaget	384	0.120	115	256	0.134	352
	9 to 12 months in lactation					
820	21	0.139	409	6	0.167	317
1235	19	0.161	235
1247	20	0.129	761
1265	20	0.145	53
1268	24	0.149	571
1269	18	0.122	54	5	0.121	265
1273	24	0.165	165	11	0.270	4518
1274	38	0.113	47
1275	12	0.190	676
1278
1279	16	0.141	809
1282	36	0.139	81
1294
1298	24	0.119	22
1418	16	0.099	61	3	0.108	103
1434	3	0.135	151	29	0.149	361
1441
1445	17	0.109	188	2	0.177	1748
1472	8	0.107	36	7	0.107	39
1473	10	0.104	45	5	0.095	40
1474	20	0.122	161	8	0.183	1280
1476	30	0.115	105
1478	8	0.104	52	16	0.126	257
1483	12	0.119	104	12	0.130	218
1488	14	0.106	97
1489	10	0.116	18	8	0.110	38
1490	16	0.100	20
Total or averaget	420	0.128	150	128	0.148	643

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

TABLE 1—(Continued)

Cow No.	More than 12 months in lactation					
	Hand*			Machine*		
	Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
	<i>number</i>	<i>per cent</i>	<i>thousands</i>	<i>number</i>	<i>per cent</i>	<i>thousands</i>
820	15	0.178	1204
1235
1247	8	0.177	859
1265
1268
1269	21	0.157	451
1273
1274
1275
1278
1279
1282
1294
1298
1418
1434
1441
1445
1472	9	0.142	328
1473
1474
1476
1478
1483
1488
1489
1490	4	0.098	18	4	0.104	435
Total or averaget	25	0.147	382	36	0.163	823
Summary for entire period						
820	66	0.123	153	57	0.153	500
1235	52	0.137	121	26	0.180	885
1247	26	0.125	80	45	0.122	218
1265	34	0.140	110	48	0.104	64
1268	124	0.129	205	24	0.138	426
1269	82	0.123	215	77	0.117	213
1273	88	0.138	119	71	0.156	700
1274	106	0.112	52	46	0.106	216
1275	64	0.148	293	31	0.186	958
1278	20	0.122	45	48	0.140	240
1279	20	0.103	44	71	0.112	305
1282	148	0.119	37	44	0.145	695
1294	61	0.119	117	20	0.126	397
1298	48	0.116	29	68	0.135	199
1418	16	0.099	61	19	0.110	397
1434	74	0.128	96	67	0.141	206
1441	9	0.106	73	32	0.117	341
1445	69	0.105	85	105	0.101	163
1472	40	0.103	40	38	0.110	184
1473	40	0.097	123	61	0.109	639
1474	84	0.116	179	101	0.125	630
1476	99	0.111	126	19	0.104	1004
1478	8	0.104	52	67	0.108	196
1483	60	0.108	73	31	0.123	160
1488	67	0.098	45	29	0.097	316
1489	32	0.100	21	18	0.108	87
1490	34	0.096	106	49	0.103	225
Total or averaget	1571	0.118	112	1312	0.124	368

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

The figures at the bottom of table 1, giving average results for all of the cows in this group, show that in every stage of lactation machine milking resulted in higher leucocyte counts and chloride values than did hand milk-

TABLE 2

The effect of machine milking versus hand milking on the leucocyte count and chloride content of milk from infected quarters

Cow No.	Infecting organism	First 3 months of lactation					
		Hand*			Machine*		
		Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
		number	per cent	thousands	number	per cent	thousands
840	<i>Streptococcus</i>	15	0.168	2931	9	0.169	4331
859	<i>Streptococcus</i>	8	0.175	4984
882	<i>Streptococcus</i>	12	0.182	5823
1235	<i>Streptococcus</i>	5	0.150	5730	13	0.235	5083
1269	<i>Ps. aeruginosa</i>	7	0.118	3375	4	0.148	3822
1445	<i>Streptococcus</i>	11	0.114	2288	11	0.138	4290
1472	<i>Streptococcus</i>	10	0.121	573	6	0.116	1692
1473	<i>Ps. aeruginosa</i>	10	0.122	1230	11	0.178	9355
1479	<i>Ps. aeruginosa</i>	8	0.093	1860
Total or average†		78	0.137	2874	62	0.173	5169
3 to 6 months in lactation							
840	<i>Streptococcus</i>	6	0.175	1901	21	0.171	2895
859	<i>Streptococcus</i>	2	0.211	5140	14	0.180	5668
882	<i>Streptococcus</i>	6	0.176	1702	6	0.199	8157
1235	<i>Streptococcus</i>	5	0.135	141	19	0.180	4889
1269	<i>Ps. aeruginosa</i>	5	0.113	5022	2	0.159	17500
1445	<i>Streptococcus</i>	15	0.121	940	9	0.158	2310
1472	<i>Streptococcus</i>	10	0.125	370	20	0.120	2524
1473	<i>Ps. aeruginosa</i>	10	0.133	6337	23	0.170	10329
1479	<i>Ps. aeruginosa</i>	7	0.101	785	2	0.133	2975
Total or average†		66	0.134	2188	116	0.164	5446
6 to 9 months in lactation							
840	<i>Streptococcus</i>	3	0.177	1129	12	0.209	5723
859	<i>Streptococcus</i>	13	0.127	1031	3	0.207	3273
882	<i>Streptococcus</i>	15	0.142	2054
1235	<i>Streptococcus</i>	4	0.118	77	6	0.245	6407
1269	<i>Ps. aeruginosa</i>	5	0.108	801	5	0.153	8150
1445	<i>Streptococcus</i>	8	0.139	562	6	0.215	2521
1472	<i>Streptococcus</i>	12	0.137	1065	16	0.127	3519
1473	<i>Ps. aeruginosa</i>	10	0.166	7370	18	0.135	12346
1479	<i>Ps. aeruginosa</i>	6	0.112	2625	8	0.141	2754
Total or average†		76	0.144	2142	74	0.166	6397

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

TABLE 2—(Continued)

Cow No.	Infecting organism	More than 9 months in lactation					
		Hand*			Machine*		
		Samples	Average chlorides	Average leucocytes	Samples	Average chlorides	Average leucocytes
		number	per cent	thousands	number	per cent	thousands
840	<i>Streptococcus</i>	3	0.181	2276
859	<i>Streptococcus</i>	6	0.166	1215
882	<i>Streptococcus</i>	6	0.207	2701
1235	<i>Streptococcus</i>	6	0.157	321
1269	<i>Ps. aeruginosa</i>	13	0.164	9548	17	0.145	4511
1445	<i>Streptococcus</i>	4	0.176	6417
1472	<i>Streptococcus</i>	18	0.146	1219	18	0.120	1479
1473	<i>Ps. aeruginosa</i>	20	0.144	6850
1479	<i>Ps. aeruginosa</i>	6	0.153	8531	8	0.180	6692
Total or average†		79	0.158	4877	46	0.144	3558
Summary for entire period							
840	<i>Streptococcus</i>	24	0.171	2448	45	0.181	3895
859	<i>Streptococcus</i>	21	0.170	1671	25	0.181	5162
882	<i>Streptococcus</i>	39	0.169	3259	6	0.199	8157
1235	<i>Streptococcus</i>	20	0.142	1577	38	0.209	5195
1269	<i>Ps. aeruginosa</i>	30	0.136	5896	28	0.148	5990
1445	<i>Streptococcus</i>	38	0.129	1827	26	0.163	3196
1472	<i>Streptococcus</i>	50	0.134	883	60	0.121	2392
1473	<i>Ps. aeruginosa</i>	50	0.142	5727	52	0.160	10821
1479	<i>Ps. aeruginosa</i>	27	0.113	3234	18	0.157	4529
Total or average†		299	0.144	3066	298	0.163	5333

* In the comparisons between hand and machine milking the data usually represent different lactation periods; in some instances, however, a cow was changed from one mode of milking to the other during a lactation period.

† Weighted average.

ing. With some exceptions the results for each individual cow also show higher leucocyte and chloride levels when milking machines were used. With cow 1,269 the differences are so small as to be insignificant. On the other hand, certain cows, such as 1,235 and 1,275, show much higher results on machine milking than on hand milking. The results on cows such as those two tend to exaggerate the group average figures for machine milking.

Summary figures on cow 1,265, shown in the section on the extreme right of table 1, indicate that the leucocytes and chlorides in the milk were lower while she was on machine milking than they were while she was on hand milking. The figures for the different stages of lactation show, however, that for 1,265 all of the results on machine milking were obtained during the first six months of lactation and all of the results on hand milking were obtained during the last six months of lactation. That lack of direct comparison possibly accounts for the apparently lower results with machine

milking. This surmise is borne out by the group average figures at the bottom of table 1, which show that with both methods of milking there is a steady increase in leucocytes and chlorides as lactation advances.

THE EFFECT OF THE METHOD OF MILKING ON INFECTED QUARTERS

Table 2 shows the results for the infected quarters of 9 cows that were on both hand and machine milking after the onset of infection. Of a total of 597 samples reported here, 299 were taken while the cows were on hand milking and 298 while they were on machine milking.

As would be expected, the leucocyte counts and chloride values in these samples were distinctly higher than those reported in table 1 for the samples from uninfected quarters. The variations found in table 2 are greater than those found in table 1 and there is less tendency with these infected quarters for the leucocytes and chlorides to rise with advancing lactation.

In general, the results in table 2 indicate that with infected quarters, as with uninfected quarters, the leucocyte counts and chloride values are higher when the cows are on machine milking than when the cows are on hand milking.

RELATION OF THE METHOD OF MILKING TO THE OCCURRENCE OF INFECTION

Whenever a newly infected quarter was found during this investigation an attempt was made to correlate the onset of the infection with the method of milking that was being used at the time. Infections that occurred during the dry period and a few infections that occurred at about the time a cow was changed from one method of milking to the other were not ascribed to either method of milking.

Of 23 streptococcal infections, the onset of which could be correlated with the method of milking, 9 occurred in cows on hand milking and 14 in cows on machine milking. During the period that these infections occurred, the numbers of cows on the two methods of milking were approximately the same. Since the total number of traceable cases was small, it is doubtful whether the difference between the 9 cases occurring on hand milking and the 14 cases occurring on machine milking is significant.

On the other hand, infections with *Pseudomonas aeruginosa* are rather definitely associated with machine milking. The onset in 29 cases of *Ps. aeruginosa* can definitely be traced to periods when the affected cows were on machine milking. Only one case has been found that appears to have originated when the cow was on hand milking. The evidence with respect to several doubtful cases is that they, also, probably are associated with machine milking.

Infections associated with organisms other than streptococci and *Ps. aeruginosa* were too few to be of significance in this study.

DISCUSSION

At the time this investigation was under way little attention was given to the length of time that the machines were left operating on the cow. The general practice was to remove the teat cups as soon as milk flow ceased, but when it was known that a cow was not letting her milk down, the machine was left on the cow for an indefinitely longer period in an effort to accomplish complete milking. According to Petersen (5) this practice will ultimately make the cow a stripper. Petersen further asserts that to leave the machine on the cow after the milk has ceased flowing may injure the lining of the teat cistern.

Dahlberg (1) has shown that cows on machine milking remain in high production longer and give milk with fewer leucocytes if the machines are left on the cow a maximum of 4 or 5 minutes at a milking. Some of the manufacturers of milking machines are now recommending an interval of milking time not to exceed 5 minutes, insisting that slow milking cows can be trained to fast milking with benefit to both production and the health of the udder.

It is quite probable that if, during this investigation, the practice now recommended for limiting milking time to 5 minutes had been adhered to, the difference in results between hand and machine milking would have been less than was actually found. Just how much less is purely conjecture. It is apparent that under the conditions of this experiment the use of milking machines caused some udder irritation and contributed significantly to the spread of *Ps. aeruginosa* infections. The relationship between the use of milking machines and the incidence of streptococcic infections in the herd is not definite.

SUMMARY AND CONCLUSIONS

The effect of milking machines on cows has been studied by comparing the results of leucocyte counts and chloride titrations of the milk when the cows were on machine milking with the results found when the same cows were on hand milking. Data for infected and uninfected quarters were studied separately. A total of 31 cows was included in the two groups.

Under the conditions of this experiment the milk of most of the cows when on machine milking yielded higher leucocyte and chloride values than when they were on hand milking. This was true for both infected and uninfected quarters. There were marked differences between cows in their response to machine milking.

Milking machines apparently contributed to the spread of *Ps. aeruginosa* infections in the herd. Whether they were a factor in the spread of streptococcic infections is not definite.

The influence of the interval of time that the machines were left on the cows as a factor in causing the high leucocyte and chloride levels with machine milking is discussed.

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AMMONIATED SUGAR BEET PULP AS A NEW NITROGENOUS FEED FOR RUMINANTS

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It has been shown (9, 10) that sugar beet pulp can be easily ammoniated to produce a palatable product containing 4 to 5 per cent nitrogen. The added nitrogen is stable and mostly in a water-soluble form. Studies (11) on pilot plant ammoniation of the dried pulp indicate that commercial ammoniation would be a relatively simple and inexpensive process. Since considerable evidence (3, 4, 5, 13) has accumulated to show that ruminants can use urea and ammonium bicarbonate for their maintenance and growth it seemed desirable to learn whether ruminants can also use ammoniated pulp for their nitrogenous nutrition. The experimental work reported in this paper was designed to give information on that point.

The term ammoniated pulp as used in this paper refers to a plain dried sugar beet pulp which has been treated with ammonia (11) to raise its nitrogen content. Likewise, ammoniated molasses pulp refers to a molasses beet pulp which has been treated with ammonia to increase its nitrogen content. Molasses pulp is a product usually made by mixing molasses with wet beet pulp at the beginning of the drying process.

On May 12, seven Holstein male calves weighing 125 to 187 pounds were placed on a milk and calf meal diet. These animals developed scours soon thereafter which held them back somewhat. They were fed milk, calf meal, alfalfa and molasses beet pulp from May 12 to July 26 with the animals receiving little milk the last month.

At the beginning of the experimental period the animals were placed in separate specially prepared pens inside an enclosed hay barn. Each pen was further protected during the coldest part of the winter and liberal amounts of pine shavings were used as bedding. Even so, it was very cold in these pens during the subzero weather. The animals were allowed to run in a vegetation-free paddock when the weather would permit.

The ammoniated pulps were prepared by adding 300 pounds of pulp to the closed ammoniation unit. Fifteen pounds of ammonia were added and the unit was revolved for thirty minutes. The temperature became 130° C. and a product containing 25–27 per cent protein ($N \times 6.25$) was obtained.

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Unammoniated pulp was then mixed with the ammoniated to give the necessary protein value for the rations.

On July 26, the animals were placed on the experimental rations shown in table 1 and each animal was referred to by the ration he ate. The rations were essentially alike except for the nitrogen source and the fact that starch was substituted for nitrogen free molasses in some cases.

TABLE 1
The composition of the basal ration

Ingredients	Protein		
	lbs.	%	lbs.
Plain dried beet pulp	60.00	9.2	5.52
Timothy hay	16.00	8.0	1.28
Starch	8.79	0.0	0.00
Molasses	12.00	0.0	0.00
Steamed bone meal	2.00	7.0	0.14
Iodized salt	1.00	0.0	0.00
Fortified fish oils	0.21	0.0	0.00
	100.00	6.94	6.94

Calves 1 and 2 received the basal ration. This contained 6.94 protein ($N \times 6.25$). Rations 1 and 2.

Calf 3 received the basal ration modified by substituting ammoniated pulp for plain pulp and starch for molasses. Total protein ($N \times 6.25$) was 12.42 per cent. Ration 3.

Calf 4 received the basal ration modified by substituting ammoniated pulp for plain pulp. Total protein ($N \times 6.25$) was 12.42 per cent. Ration 4.

Calf 5 received the basal ration modified by substituting ammoniated pulp for plain pulp and starch for molasses. Total protein ($N \times 6.25$) was 17.02 per cent. Ration 5.

Calf 6 received the basal ration modified by substituting ammoniated pulp for plain pulp. The protein ($N \times 6.25$) was 17.02 per cent. Ration 6.

Calf 7 received the basal ration except toasted soybean meal was substituted for the starch and molasses. Total protein ($N \times 6.25$) was 16.42 per cent. Ration 7.

Calf 8 received the basal ration except molasses ammoniated pulp was substituted for plain pulp. Total protein ($N \times 6.25$) was 17.02 per cent. Ration 8.

Molasses was excluded from rations 3 and 5 to note whether this soluble carbohydrate promoted the use of the nitrogen in the ammoniated pulp.

The calves were fed equal amounts of feed three times daily and sufficient fortified fish oils were added to each feeding to supply each animal daily with 2700 units of vitamin A and 500 units of vitamin D per 100 pounds of body weight. Individual weights were taken weekly

during the experimental periods and the increase was recorded as growth. The animals were weighed daily the last three days and the mean was taken as the final value.

Pictures were taken of the animals 132 and 222 days from the beginning of the experiment. Calves 1 and 2 were taken from the basal diet and placed on rations 8 and 5 respectively, 150 days from the beginning of the experiment. The second pictures were taken 72 days after the protein deficient animals had been changed to diets containing ammoniated pulp.

Blood was taken from the jugular vein of each animal 97, 140 and 210 days after the beginning of the experiment. It was analyzed for total erythrocytes, hemoglobin, total leucocytes, neutrophiles, eosinophiles, monocytes and lymphocytes by the usual clinical methods. The color index was then calculated by the usual procedure. The amounts of glucose, calcium, total serum protein, non-protein nitrogen, urea, cholesterol, serum albumin and serum globulin were determined (6, 7) and the albumin-globulin ratio was calculated.

The steers were slaughtered at the Swift and Company yards under federal inspection and the liver from No. 7 and a kidney from each animal were sent to the pathological laboratory for further investigation.

The animal tissues were analyzed (1) for total protein, water-soluble protein, coagulable protein and moisture. The A.O.A.C. method was modified for the water-soluble and coagulable-proteins on the livers and kidneys as follows:

Ten to thirteen grams of freshly ground sample was exhausted with 10 cc. of water at room temperature. The sample was transferred to a 250-cc. volumetric flask with water and diluted almost to the mark and stored in the refrigerator 65 hours at 2° C. Samples were removed from the refrigerator in pairs and made up to volume immediately. They were mixed well and the entire 250 cc. was centrifuged thirty minutes. The supernatant portion was decanted and 25-cc. room-temperature aliquots were used for water-soluble proteins and 75-cc. for coagulable proteins. One cc. of 0.1 N acetic acid was added to the 75 cc. and the sample was boiled five minutes to coagulate the proteins. The coagulum was transferred to a filter and washed with 250 cc. of hot water for the livers and 25 cc. hot water for the kidneys. Nitrogen was determined by the Kjeldahl method and protein was calculated ($N \times 6.25$).

EXPERIMENTAL RESULTS

It was not necessary to work the animals on to these feeds for the palatability was such that they ate well from the beginning except for the first animal on ration 6. He did not clean his feed up readily until he was placed upon ration 4 later in the experiment. Each animal consumed 5 pounds of feed early in the experiment. This was increased from time to

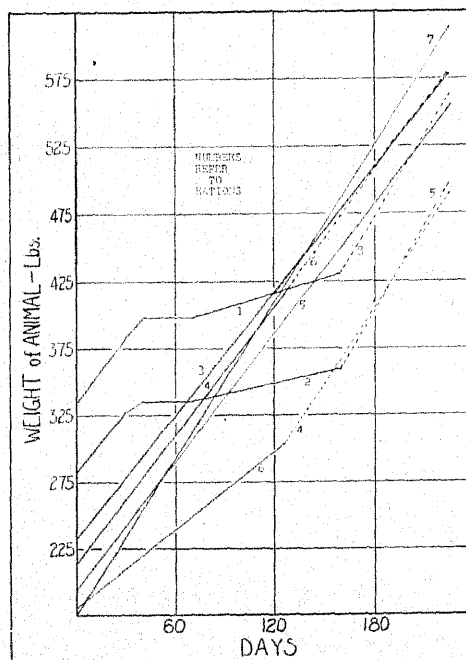


FIG. 1. Growth of male calves—225 days.

time until after 34 days 12 pounds were being eaten. After 70 days the amount was increased to 15 pounds. This proved to be too much so the amount was decreased to 11 pounds for the rest of the experiment. The

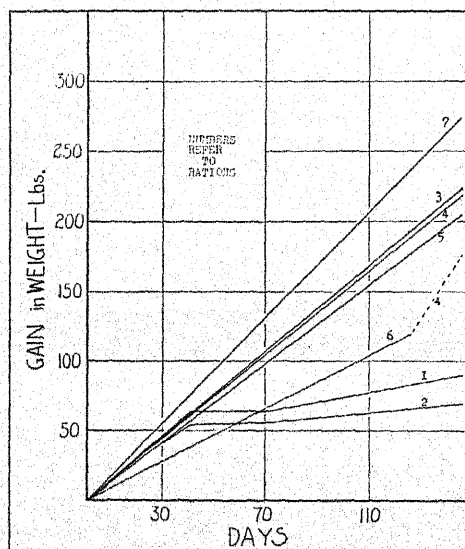


FIG. 2. Growth of male calves—146 days.

appetites were good and equalized feeding was practiced throughout without difficulty. No digestion troubles of any kind developed. There was no evidence of diuresis in any of the animals. The growth of the animals is shown in figures 1, 2 and 3. Figure 1 shows the weight of the animals during the experiment (225 days). Figure 2 shows the gain in weight after 146 days and figure 3 shows the gain in weight at the end of 225 days.

The animals on rations 1 and 2 continued to grow quite rapidly during the first 40 days of the ration but began to develop protein deficiency symptoms

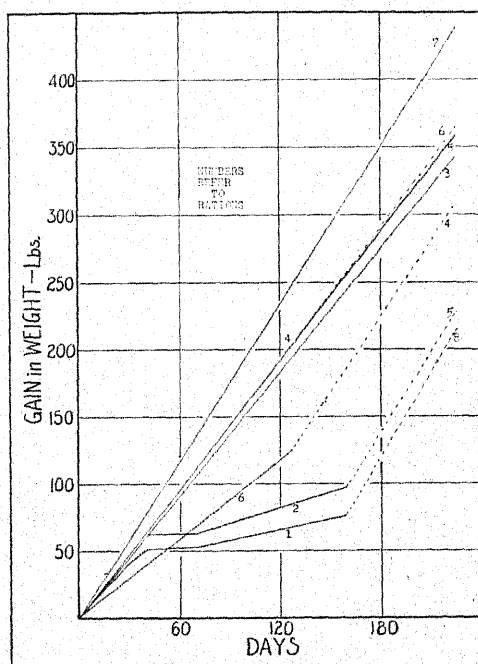


FIG. 3. Growth of male calves—225 days.

at the end of 30 days. Then they did not grow at all for 30 days, after which they grew very slowly until they were placed on rations 8 and 5, respectively.

The protein deficiency symptoms as shown in figure 4 became very marked before they were placed on the rations containing ammoniated pulp. The animals became very thin, developed a sharp-boned hump in their back, a pot belly, a rough and shaggy coat and a listless gait.

After the protein-deficient animals were placed on rations 8 and 5 they started to lose their protuberant bellies, their backbones straightened out, their coats took on more life, they became much more lively, and began to grow at the rate of about 2 pounds per day.

There was little difference in the growth rates of animals 3, 4 and 5. Molasses was excluded from rations 3 and 5 to note whether the absence of

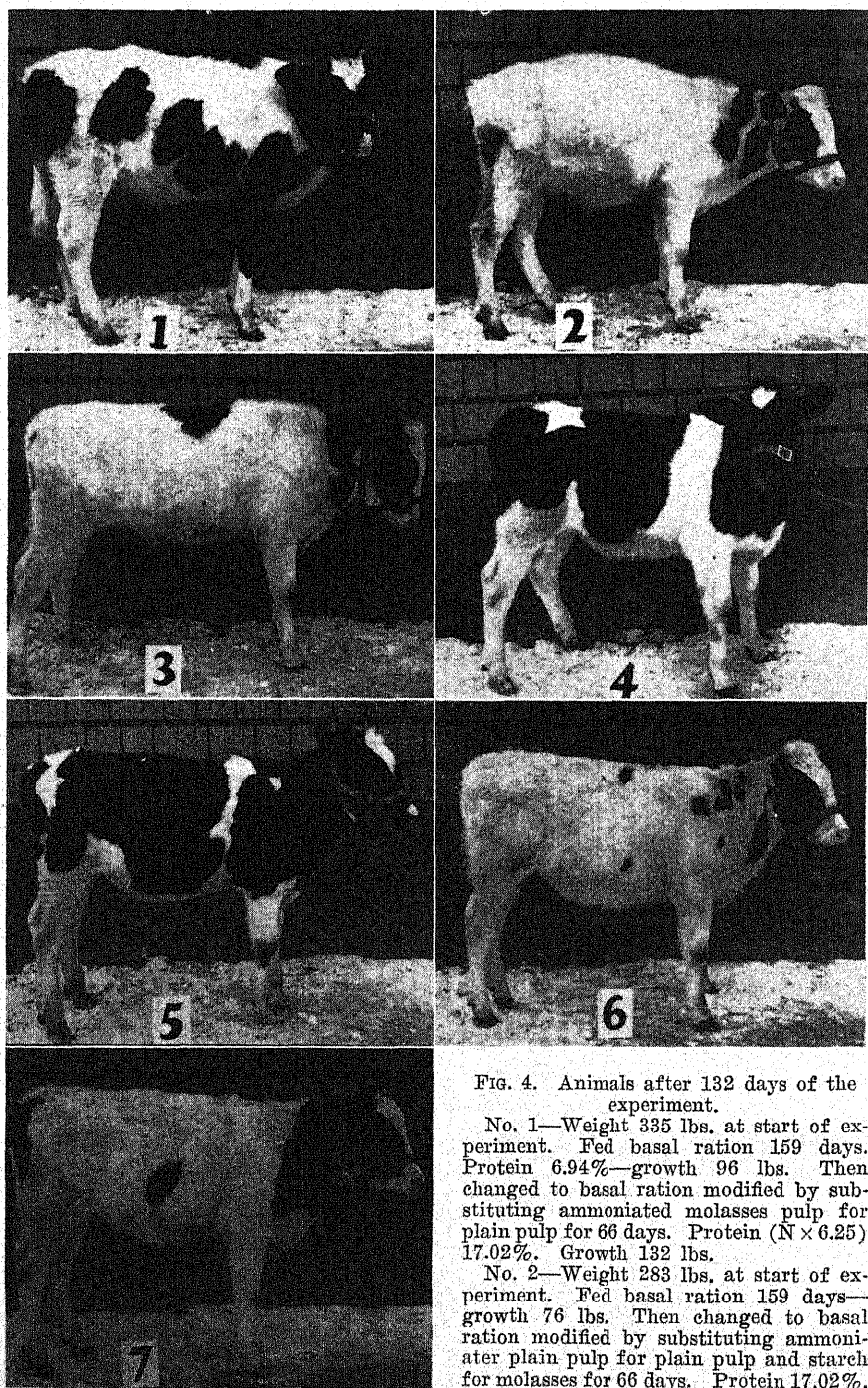


FIG. 4. Animals after 132 days of the experiment.

No. 1—Weight 335 lbs. at start of experiment. Fed basal ration 159 days. Protein 6.94%—growth 96 lbs. Then changed to basal ration modified by substituting ammoniated molasses pulp for plain pulp for 66 days. Protein ($N \times 6.25$) 17.02%. Growth 132 lbs.

No. 2—Weight 283 lbs. at start of experiment. Fed basal ration 159 days—growth 76 lbs. Then changed to basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses for 66 days. Protein 17.02%. Growth 138 lbs.

this source of soluble carbohydrate inhibited the use of the nitrogen in the ammoniated pulp or conversely whether its presence promoted nitrogen utilization over that of starch alone. The results indicate no difference in the growth of the animals on rations containing all starch as compared to those in which molasses replaced part of the starch.

The animal on ration 6 grew very slowly during the preliminary 14 weeks before the experiment was started. Furthermore, he continued to grow more slowly than animals 3, 4, 5 and 7 from the beginning of the experiment. This animal was on the ration containing molasses and pulp ammoniated to 4.16 per cent nitrogen. He did not eat it as well as the other animals were eating their rations. Therefore, after 159 days the rations of animals 4 and 6 were exchanged to learn if there was something about ration 6 to give the inferior results. After this the slow growing animal grew much faster on ration 4 while the new animal on ration 6 continued to eat and grow at its previous rate on ration 4. Apparently it was not the ration alone but probably an animal-ration relationship causing the poor initial results from ration 6. Growth was just as good on rations containing 12.42 per cent protein as on those containing 17.02 per cent protein.

The pictures of the animals at the end of the experiment are shown in figure 5. They show that the animals on ammoniated pulp grew during the 90-day interval between pictures and that the physical condition of the protein deficient animals was greatly improved by the ammoniated pulp.

The data in table 2 show the daily gains and feed consumed per 100 pounds gain in weight for the various growing periods shown in figure 1. The soybean fed animal gained 1.956 pounds per day for 225 days. Ration one provided only 14.26 per cent of this growth for animal 1; ration two, 10.38 per cent; ration three, 78.07 per cent; ration four, 81.80 and 95.55 per cent; ration five, 81.34 and 106.90 per cent and ration six, 49.89 and 84.15 per cent. The larger value on ration 5 was obtained when it was fed to animal 2 that had been on the protein deficient diet. Animal 4 did about equally well on rations 4 or 6 while animal 6 grew much better on ration 4 than ration 6. The feed consumed per 100-pound gain as compared to those listed by Morrison (14) must be considered good.

No. 3—Initial weight 234 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses. Protein 12.42%. Growth after 159 days 244 lbs.; after 225 days 343 lbs.

No. 4—Initial weight 214 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp. Protein 12.42%. Growth after 159 days 261 lbs.; after 225 days 364 lbs.

No. 5—Initial weight 195 lbs. Fed basal ration modified by substituting ammoniated plain pulp for plain pulp and starch for molasses. Protein 17.02%. Growth after 159 days 255 lbs.; after 225 days 358 lbs.

No. 6—Initial weight 181 lbs. Fed basal ration modified by substituting ammoniated pulp for plain pulp for 126 days. Protein 17.02%. Growth 123 lbs. Then changed to ration four for 99 days. Growth 185 lbs.

No. 7—Initial weight 174 lbs. Fed basal ration modified by substituting toasted soybean meal for starch. Protein 16.42%. Growth after 159 days 313 lbs.; after 225 days 440 lbs.

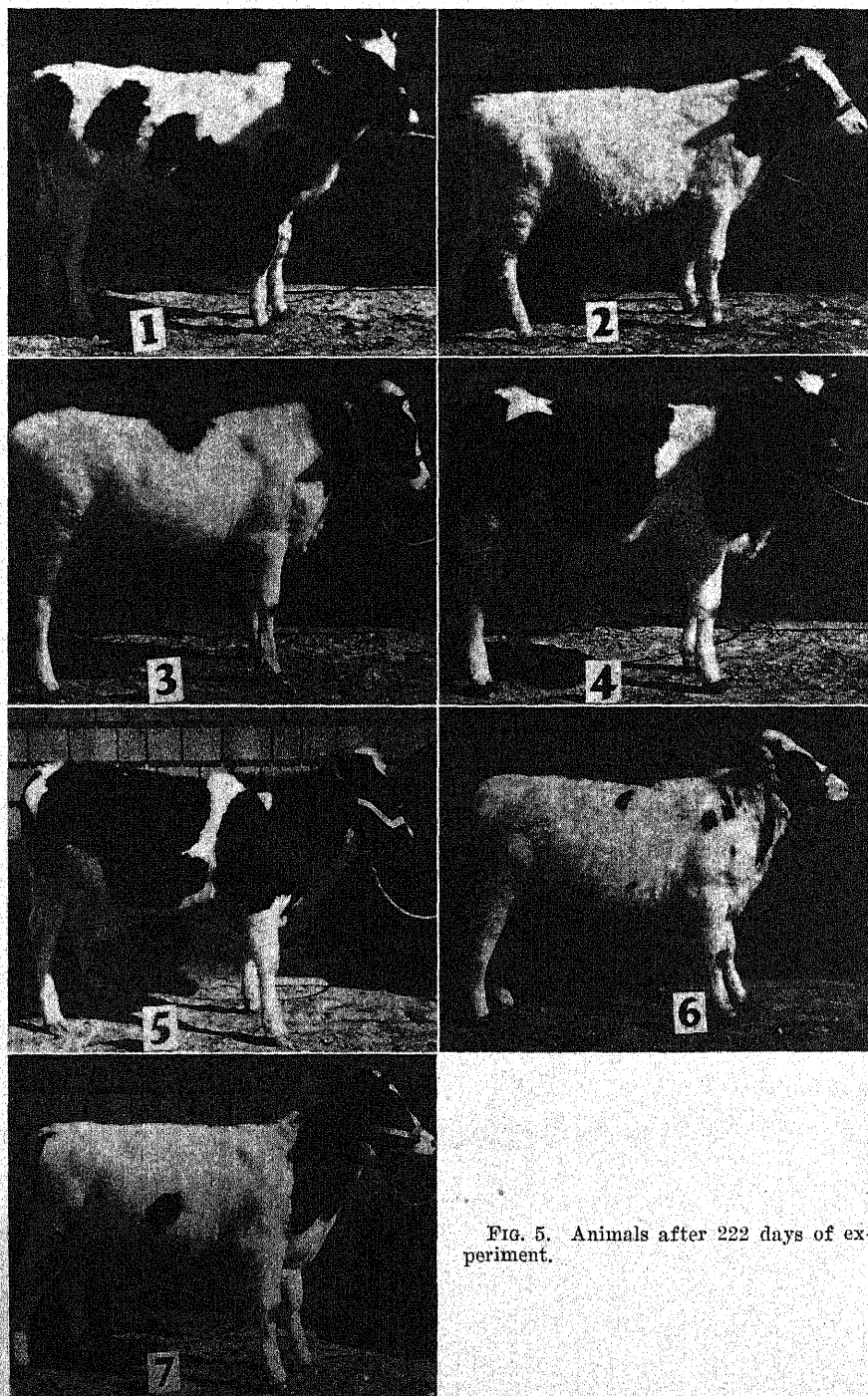


FIG. 5. Animals after 222 days of experiment.

ANALYSES OF THE BLOOD

The data on the blood are shown in table 3. The first two blood samplings were taken while animals 1 and 2 were still on the deficient rations. The third samples were taken near the end of the experiment and after animals 1 and 2 had been changed to the ammoniated rations and had gained rapidly in weight. Thus, blood from animals on ammoniated pulp was obtained from the third sampling of animals 1 and 2 and from all three samplings from animals 3, 4, 5 and 6.

The blood from animal No. 7 represents that from an animal on a conventional protein source. The approximate normal value (2) for cattle

TABLE 2

Rate of gain and amount of feed consumed by steers during certain feeding periods

Steer No.	Ration No.	Experimental period	Daily gain	Portion of daily gain promoted by soybean meal	Total feed consumed	Feed consumed per 100 lbs. body weight
		<i>days</i>	<i>lbs.</i>	<i>%</i>	<i>lbs.</i>	<i>lbs.</i>
1	Basal	41-159	0.279	14.26	1463	4433
	Ration 8	159-225	2.000	102.25	726	550
2	Basal	41-159	0.203	10.38	1463	6096
	Ration 5	159-225	2.091	106.90	726	526
3	Ration 3	1-225	1.527	78.07	2545	742
4	Ration 4	1-126	1.600	81.80	1456	728
	Ration 6	126-225	1.646	84.15	1089	667
5	Ration 5	1-225	1.591	81.34	2545	711
6	Ration 6	1-126	0.976	49.89	1456	1184
	Ration 4	126-225	1.869	95.55	1089	589
7	Ration 7 (Soybean meal)	1-225	1.956	100.00	2545	579

listed in table 3 represents mean values and are largely based on adult cattle. Miller (12) has shown that the number of erythrocytes decreases in calves as they mature while the hemoglobin values remain quite constant with wide differences in individual animals. He shows that calves appear to have more leucocytes than the adult with the proportions of the kinds of leucocytes remaining about the same as in adults. He also shows that young bovine animals have less serum protein than adults. Hodgson *et al.* (8) show that the glucose in the blood of dairy animals expressed in milligrams per 100 cc. of blood was 100.4, 88.2, 80.2, 75.4, 69.6, 67.8, 62.2 and 55.0 after 6 days, 4 weeks, 3, 7, 11, 15, 19 and 23 months, respectively.

The data show the total erythrocytes to be normal in all cases. The third sample from animal No. 7 was somewhat higher than the other values. The hemoglobin was normal in all samplings and the total leucocytes were about the same except that the first two samplings on the soybean-meal animal were high. Perhaps the abnormal leucocyte and erythrocyte values on the soy-

TABLE 3
Composition of blood of growing Holstein steers

Steer No.	Ration No.	Blood sampling date	Erythrocytes		Color index	Leucocytes				
			Total count per cu. mm.	Hemo-globin		Total count per cu. mm.	Neutro-philles (adults)	Eosino-philles	Mono-cytes	Lympho-cytes
1	1	1*	7,450,000	9.7	0.42	12,700	14	4	82
	1	2	8,560,000	9.8	0.37	12,000	29	1	70
	8	3	7,930,000	9.7	0.40	8,500	23	77
2	2	1	7,550,000	9.7	0.40	6,600	26	1	1	72
	2	2	9,240,000	9.7	0.34	6,000	18	1	81
	5	3	7,810,000	9.2	0.40	9,300	11	1	2	86
3	3	1	8,850,000	12.2	0.45	8,800	27	1	73
	3	2	7,410,000	10.7	0.47	11,050	24	4	1	70
	3	3	7,600,000	9.2	0.40	10,700	29	70
4	4	1	9,630,000	10.6	0.36	8,700	34	1	1	64
	6	2	8,480,000	9.2	0.36	7,800	27	73
	6	3	8,700,000	10.7	0.40	12,200	27	73
5	5	1	9,600,000	10.3	0.35	4,800	23	1	2	64
	5	2	9,190,000	10.7	0.35	7,200	24	2	74
	5	3	8,840,000	10.7	0.39	7,900	28	72
6	6	1	9,650,000	11.2	0.40	6,300	27	2	71
	4	2	9,370,000	9.8	0.34	8,250	22	1	1	76
	4	3	9,100,000	10.6	0.37	13,700	31	69
7	7	1	7,500,000	9.4	0.40	23,900	25	1	74
	7	2	8,630,000	9.9	0.32	22,200	27	3	70
	7	3	11,560,000	11.7	0.33	10,100	46	54
Approximate normal for cattle (2)			6,325,000	10.3	7,900†	21	5	10	64

TABLE 3—(Continued)

Steer No.	Ration No.	Blood sampling date	Glucose	Calcium	Cholesterol	Non-protein nitrogen	Urea N	Total serum protein	Serum		Albumin/globulin ratio
									Albumin	Globulin	
			mgms./100 cc. blood	mgms./100 cc. serum	mgms./100 cc. serum	mgms./100 cc. blood	mgms./100 cc. blood	per cent			
1	1	1*	74.0	13.51	88	18.0	11.0	6.56	3.94	2.62	1.50/1
	1	2	69.5	9.70	127	26.9	9.2	5.60	3.51	2.09	1.68/1
	8	3	83.0	10.00	81	32.0	15.4	4.38	2.48	1.90	1.31/1
2	2	1	73.0	12.93	58	16.0	7.8	7.88	4.28	3.60	1.19/1
	2	2	74.5	10.70	121	7.1	5.66	3.51	2.15	1.63/1
	5	3	94.0	10.60	62	33.5	11.2	5.05	3.00	2.05	1.46/1
3	3	1	45.0	12.73	44	26.0	9.2	7.98	4.52	3.46	1.31/1
	3	2	82.0	10.90	101	34.1	9.3	5.49	3.85	1.64	2.34/1
	3	3	89.0	10.50	69	32.0	8.1	5.22	3.55	1.67	2.13/1
4	4	1	77.0	12.42	55	24.8	9.0	5.49	3.94	1.55	2.54/1
	6	2	77.5	11.10	85	33.3	10.0	4.76	3.32	1.44	2.31/1
	6	3	97.0	11.10	71	29.5	9.1	6.42	3.42	3.00	1.14/1
5	5	1	65.0	11.31	58	30.0	16.3	6.18	4.10	2.08	1.97/1
	5	2	81.0	10.60	81	26.5	15.1	5.45	4.15	1.30	3.19/1
	5	3	89.0	9.60	72	32.0	16.0	5.33	3.45	1.88	1.84/1
6	6	1	55.0	11.92	66	32.0	14.6	6.49	4.09	2.40	1.70/1
	4	2	75.0	10.90	61	36.6	11.3	4.83	3.36	1.47	2.29/1
	4	3	86.0	10.10	75	32.0	15.1	5.15	3.64	1.51	2.41/1
7	7	1	73.0	11.92	70	30.4	14.4	6.67	4.28	2.39	1.79/1
	7	2	78.5	11.10	118	29.8	15.1	5.62	3.47	2.15	1.61/1
	7	3	92.0	11.10	106	34.5	17.0	5.67
Approximate normal for cattle (2)			40-60	9-12	50-230	20-40	6-27	4-8.5	7.2

* 1—Oct. 30, 1942.

2—Dec. 12, 1942.

3—Feb. 20, 1943.

† 5,000-12,000 (12).

bean-meal-fed animal were associated with the diffuse cirrhotic liver found in the animal when slaughtered. The adult neutrophils were much the same in all cases except in the last blood samples from the soybean-fed animal.

The lymphocytes were much the same in all animals.

The glucose values were similar in all animals and while slightly above that listed as normal for the adult they must be considered normal for animals of this age. Feed was never withheld before taking the blood samples, for Hodgson (*loc. cit.*) shows that this has no effect on the blood sugar of the bovine. Carbohydrate metabolism appeared to be functioning normally.

The calcium content of the blood fell between the normal ranges in all samplings and was remarkably constant. All the cholesterol values but one fell within what is listed as normal for cattle.

The non-protein and urea nitrogen of the blood was low during the period the animals were on the protein-deficient diet. The third sampling, which was taken after these animals were placed on diets containing more nitrogen, showed an increase in non-protein nitrogen and urea. All animals were metabolizing nitrogen in a normal manner. It is interesting to note that in case of the animals on ration 3 and 4 the urea values were less than one-third of the non-protein nitrogen values while the urea values for the blood of the animals on ration 5, 6 and 7 are almost half those of the non-protein values. The non-protein values are much the same whether the ration was 12.4 or 17.02 per cent protein while the urea values were about 1.5 times larger for the rations having the higher protein values.

Total serum protein was normal and much the same in all samplings. The serum albumin and globulin values for the animals on the ammoniated pulp diets were similar to those of the control animal and confirm the work of Miller (12) that serum protein is lower in young animals than in adults. The albumin-globulin ratios were similar in all cases.

The animals were slaughtered at Swift and Company under federal supervision. All animals and internal organs were pronounced normal by the federal inspectors except the liver in animal No. 7. This liver showed a diffuse cirrhosis. Macroscopic examinations of one kidney from each animal showed each to be a normal healthy kidney.

The carcasses were held in the coolers for 48 hours after the animals were slaughtered. A rib steak was then taken from each animal and tested for color and flavor at the Swift and Company Laboratories. The meat from each animal was found to be normal in color and flavor. Section of the ribs were taken from each animal and roasted and found to be normal in flavor, color and odor.

Table 4 shows the weight of the animals before they went into the coolers and the dressing percentage. The animals were not in a good grade of finish for the experiment was not designed for this purpose. However, a very small amount of fat was deposited on the inside of the ribs and they brought

TABLE 4

The weight of the animals when dressed and before going into the coolers

Animal No.	Dressed wt. before going into cooler	Dressing percentage*
	<i>lbs.</i>	
3	300	52.00
4	299	51.73
5	278	50.27
6	254	51.94
7	339	55.21

* Based on feed lot wt.

\$10.25 per hundred weight. The soybean animal had a slightly higher dressing percentage than the animal fed ammoniated pulp.

Part of the liver, one kidney and five ribs were taken from each carcass and analyzed for the constituents shown in table 5. Since the cuts from the different steers contained different amounts of fat some variation in the analyses from animal to animal is expected.

The results on the ribs, livers and kidneys show that the animals fed ammoniated pulp contained much the same amount of fat, total protein, water-soluble protein, coagulable protein and moisture as the soybean-fed animal. This fact, together with the growth increments, is conclusive evidence that the animals were using the ammonia added to the dried beet pulp for their protein metabolism.

TABLE 5

Composition of tissue (moisture free basis)

Animal number	Rib cut				
	Fat	Total protein	H ₂ O sol. protein	Coagulable protein	Moisture
	%	%	%	%	%
3	31.7	65.3	14.8	7.1	70.3
4	19.5	73.4	16.2	9.3	72.2
5	23.3	74.9	17.0	7.8	72.9
6	13.9	83.1	19.7	9.6	75.1
7	21.3	75.1	17.5	9.0	73.1
	Livers				
3	7.1	69.0	27.8	12.0	71.6
4	5.6	72.0	26.2	11.5	71.4
5	4.1	67.8	30.0	15.2	71.0
6	5.6	67.8	26.5	11.3	71.7
7	6.7	73.1	26.2	15.0	69.9
	Kidneys				
3	30.3	60.3	29.0	9.5	73.8
4	19.7	70.3	32.6	14.6	76.1
5	35.1	58.7	25.6	9.2	70.8
6	32.7	56.5	28.3	7.9	73.5
7	38.7	49.5	26.0	6.9	71.1

DISCUSSION OF RESULTS

Animals on the basal diet grew very little while those on the basal ration plus ammoniated pulp for 225 days grew as much as 81.34 per cent of the growth promoted by the toasted soybean meal. Animals on a low protein diet and then changed to a diet in which the nitrogen was raised only by the ammoniated pulp grew 102 and 107 per cent of that of the soybean meal animal. Ration 6 gave only about half as good results as soybean meal with one animal but gave about 84 per cent of soybean meal when fed to another animal. The animal which did so poorly on ration 6 grew 95.55 per cent as well as the soybean meal animal when placed on ration 4. The animals on the ammoniated diet grew at the rate of 1.5, 1.65, 1.59 and 1.87 pounds per day while those animals changed from the basal ration to a ration containing ammoniated pulp grew at the rate of 2.0 pounds per day. The

TABLE 6
Basal rations of three non-protein nitrogen experiments for growing Holstein calves

	After Hart <i>et al.</i> (3)		Present experiment
	Holstein males	Holstein females	Holstein males
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
Yellow corn	29.5	20.0
Dried beet pulp (plain)	60.0
Ground timothy	47.0	47.5	16.0
Starch	20.0	24.0	20.79
Corn molasses	10.0
Steamed bone meal	2.0	2.0	2.0
Salt	1.0	1.0	1.0
Cod liver oil	0.5	0.5	0.21
Total	100.0	105.0	100.0

soybean-meal-fed animal grew at the rate of 1.96 pounds per day. It is interesting to compare these results with those of the two experiments reported by Hart *et al.*, since Holstein calves were used in all three experiments and the diets were very similar except that sugar beet pulp was used in place of yellow corn and the amount of timothy varied somewhat. This comparison is shown in tables 6 and 7.

The animals on urea and ammonium bicarbonate grew at the rate of 0.8 to 1 pound per day while those on ammoniated pulp for 225 days grew at the rate of 1.53 to 1.64 pounds per day.

It has been shown (13) that ruminants are able to use non-protein nitrogen compounds because the microflora in their stomachs convert such nitrogen into bacterial protoplasm protein.

The enzymes pepsin, trypsin and peptidases then act on the proteins to liberate amino acids in the intestines where they are absorbed into the blood stream.

TABLE 7
Growth rates of Holsteins on certain nitrogenous rations

Holstein male calves ^a (Hart <i>et al.</i>)				Holstein female calves ^b (Hart <i>et al.</i>)				Holstein male calves (present experiment) ^c			
Ration				Ration				Ration			
No.	N supplement	% protein (N × 6.25)	Daily growth (290 days)	Basal	N supplement	% protein (N × 6.25)	Daily growth (365 days)	Basal	N supplement	% protein (N × 6.25)	Daily growth (225 days)
1—Basal	6.00	0.693	Basal	5.38	0.0	Basal	6.94	lbs. 0.279
2—“	Urea	18.00	1.000	“	1.4 lbs. Urea	9.49	1.0	“	A.P.*	17.02	2.000 ^d
3—“	NH ₄ HCO ₃	18.00	0.951	“	1.4 lbs. Urea	9.49	0.80	“	“	6.94	0.203
4—“	Casein	18.00	1.472	“	4.7 lbs. Casein	1.50	“	“	17.02 ^d	2.091 ^d
5—“	“	2.8 lbs. Urea	13.70	0.90	“	“	12.42	1.527
6—“	“	4.3 lbs. Urea	17.64	0.90	“	“	12.42	1.618
7—“	“	11.1 lbs. NH ₄ HCO ₃	17.04	0.90	“	“	17.02	1.591
				“	11.0 lbs. Casein	15.52	1.2	“	“	17.02	0.976
				“	“	“	12.42	1.869
				“	“	Toasted soybean meal	16.42	1.956

^a Animals were 275-375 pounds at beginning of experiment and 575-700 pounds at end of experiment.

^b “ “ 250-290 “ “ “ “ 592-678 “ “ “ “

^c “ “ 174-335 “ “ “ “ 489-614 “ “ “ “

^d Rations were changed during the experiment.

* Ammoniated pulp.

Rose *et al.* (15) have shown that the amino acids lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine are indispensable for the rat and dog. Since the animals on ammoniated pulp did not grow as well as the one on soybean meal it would appear that either all of the essential amino acids were not synthesized or not synthesized in sufficient quantity to give maximum growth. This leads to the interesting question of whether the growth promoting ability of ammoniated pulp for ruminants would be increased when fed in practical diets containing normal and multiple supplementary nitrogen sources such as barley, corn and alfalfa hay with these materials supplying less natural protein than is needed by the animal for maximum growth. Under such conditions if the growth were not maximum the question of supplying in an economical form those essential amino acids not synthesized by the ruminants, should be an interesting and fruitful study.

SUMMARY

1. An experiment on the ability of Holstein calves to grow on ammoniated plain sugar beet pulp has been conducted.
2. The experiments indicate that the animals can use such nitrogen sources for their nutritional needs. They grew at the rate of about 1.6 pounds per day as compared with 1.96 pounds for one animal on toasted soybean meal.
3. Animals fed a diet in which starch was substituted for molasses grew as well as those on rations containing the molasses. This indicated that the soluble carbohydrate did not further the use of the nitrogen by the microorganisms in the digestive tract any better than starch.
4. The animals grew just as well on rations 12.42 per cent protein as those on 17.02 per cent protein ($N \times 6.25$).
5. No diuresis of any of the animals occurred.
6. The blood was analyzed for fifteen different constituents three times during the experiment and found to be normal.
7. The animals and their internal organs passed federal inspection at the Swift and Company yards. The soybean-meal animal had a diffuse cirrhotic liver. This is not evidence that the liver condition was connected with the soybean meal. Macroscopic examinations of the kidneys showed them to be normal.
8. Rib and liver cuts as well as one kidney from each animal were analyzed and found to be normal in protein. The color and flavor of the meat was normal.
9. Further work is indicated.

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ANNOUNCEMENT
THIRTY-NINTH ANNUAL MEETING

JUNE 20-22, 1944

CALL FOR PAPERS

May we ask all members of the association who contemplate presenting papers for the consideration of the program committee to send the titles to the chairman, Professor H. P. Davis, Dairy Husbandry Department, University of Nebraska, Lincoln 1, Nebraska, at once. Titles will be accepted until April 1, but it is desired that they be received as early as possible because the program committee cannot meet and will have to carry on its work by correspondence. Unless otherwise arranged for by the committee, all papers are not to exceed 12 minutes in length and abstracts must be in the hands of the committee chairman not later than June 1.

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LIVE WEIGHT AND MILK-ENERGY YIELD IN BRITISH GOATS

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The London Dairy Show, staged normally in mid-October each year, embraces a milch goat competition based on milk and fat yields for a two-day period. Records of these competitions are published in the *Journal of the British Dairy Farmers' Association* and include live weights of the individual goats at time of the contest, as well as various data of milk yield.

The present article deals with these two-day records (on a per-day basis) for the 17 years, 1920-1937 except 1927, and is particularly concerned with the size-yield relationship.

STAGE OF LACTATION

Since goats have a pronounced tendency to bear their young in the spring season they are usually rather far advanced in stage of lactation at time of the contest in October. For present purposes all records more than 360 days after kidding are rejected (14 records, 486 to 959 days after kidding). All other records are used, a total of 318 distributed as follows, with respect to 30-day month after kidding:

Month	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th	12th
Number	1	5	5	14	28	38	85	98	40	1	2	1

Apparently kidding occurs most frequently in the month of March. The delayed stage of lactation must be kept in mind in considering the magnitude of yields. Assuming a linear lactation curve, the daily yield for the first 8 months of lactation would be somewhat greater than the daily yield for the contest period. Of course, what the yields represent is only the contest period, for the goats selected to enter the contest. Naturally a goat with too little persistency, that is, milking at too low a level at the time would not be entered.

AVERAGE PERFORMANCE BY BREEDS

Table 1 shows the average of the 318 records to be 8.36 pounds of milk and 0.390 pounds of fat per day at an average stage of lactation 195 days

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¹ On leave of absence for military service.

TABLE 1
Average data (daily basis) for goats by breeds, in order of live weight, *W*,
at the London Dairy Show

Breed	n	Stage ds.	Fat %, raw	Fat %, weighted	Milk	Fat	FCM	W	100FCM/W*
					lbs.	lbs.	lbs.	lbs.	lbs.
Toggenburg	22	185	4.08	4.09	7.33	0.309	7.44	130	5.71
Unidentified	106	185	4.86	4.76	6.51	0.309	7.25	134	5.46
Anglo Nubian Swiss	25	209	4.77	4.69	8.86	0.416	9.78	153	6.43
British Toggenburg	24	192	4.57	4.54	8.76	0.398	9.47	155	6.13
Saanen	17	213	4.72	4.66	8.48	0.395	9.31	163	5.80
Anglo Nubian	18	177	6.37	6.30	7.08	0.446	9.52	168	5.73
British Alpine	45	197	4.61	4.55	9.31	0.423	10.08	173	5.97
British	20	211	4.74	4.65	10.72	0.499	11.77	176	6.69
British Saanen	41	213	4.46	4.42	11.51	0.509	12.24	176	7.04
All breeds	318	195	4.76	4.66	8.36	0.390	9.19	154	5.99

* Averages of the individual 100FCM/W values.

after kidding. The average milk-energy yield, FCM, is 9.19 pounds of 4 per cent milk.² The average of the 318 fat percentages is 4.76 but when weighted by milk yield this becomes 4.66.

As between breeds considerable variation is shown. The breed class designated "unidentified" is largely composed of "just goats" but includes a few animals properly belonging in another class, where the breed identity could not be ascertained from the published record. The breeds are arranged in table 1 in order by average live weight. The larger size and greater productivity of the British strains (British Alpine, British, British Saanen) are conspicuous.

TABLE 2
Variance with respect to certain items, as between and as within certain groups—
London Dairy Show goats. Live weight, *W*, in pounds; milk
and FCM in pounds per day

Group	Item	Degrees of freedom		Variance		F	5% F	1% F
		Between	Within	Between	Within			
Breed	Live weight	8	309	13,289.0	643.0	20.67	1.97	2.57
"	Milk	"	"	123.1	4.4	27.78	"	"
"	FCM	"	"	128.9	5.5	23.37	"	"
"	100FCM/W	"	"	11.7	2.3	5.07	"	"
Age	Live weight	9	308	3,225.0	896.0	3.60	1.91	2.48
"	FCM	"	"	10.6	8.6	1.24	"	"
"	100FCM/W	"	"	5.2	2.5	2.08	"	"
Live weight	FCM	16	301	64.2	5.7	11.31	1.68	2.07
"	100FCM/W	"	"	3.1	2.5	1.24	"	"

² FCM = $0.4 \times \text{milk} + 15 \times \text{fat}$. One pound FCM = 340 kilocalories milk energy = 0.034 pounds milk protein regardless of the fat percentage of the natural milk. This relation is known to hold quite accurately in cows and is presumed to be applicable in goats.

LIVE WEIGHT AND YIELD

The variance data of table 2 show that the breeds are distinctly different with respect to live weight and FCM yield; less distinctly (although significantly in the statistical sense) with respect to FCM/W, that is, milk energy per unit live weight. Reference to table 1, column headed 100FCM/W, indicates the breed differences in FCM/W arise in a tendency for the larger breeds to produce more milk energy per unit live weight than the smaller breeds. Evidently the British strains are being developed for large size and milking proclivity; and milking capacity appears to be fully proportional to the larger size, as between breeds.

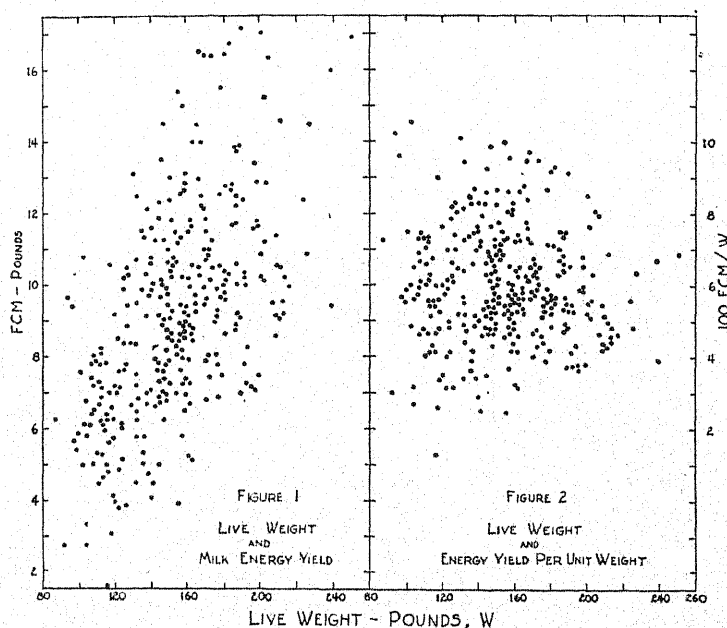


FIG. 1, FCM, in pounds per day plotted against live weight in pounds for each of the 318 records.

FIG. 2, FCM, in pounds per day per 100 pounds live weight plotted against live weight in pounds, same records as figure 1.

Figure 1 shows each of the 318 FCM records plotted against live weight without regard to breed. FCM ranges from 1.5 to 17.2 pounds per day and live weight ranges from 88 to 250 pounds. There is a fairly close correlation between live weight and milk-energy yield, amounting to $r=0.57$. If milk-energy yield is expressed as a power function of live weight, $FCM=aW^b$, it is found that $b=0.91$. From table 1 if FCM is expressed as $FCM=aW^b$ it comes out that $b=1.54$, as between breeds; and $b=0.57$ as within breed.

Figure 2 shows each of the 318 FCM/W records plotted against live weight. If FCM is proportional to $W^{0.91}$ (figure 1) it should follow that FCM/W is proportional to $W^{-0.09}$, that is FCM/W decreases slightly with increasing W. Table 2 shows, however, that FCM/W variance as between live weight groups differs only slightly from the variance within live weight groups. In other words FCM/W appears to be independent of W in the 318 records taken as a whole.

DISCUSSION

There are some elements of uncertainty as to the broad meaning of the present size-yield data. The goats entered in the contest are a selected lot, to meet the competition which is predominantly one of points for production, viz.: 1 point per pound of milk plus 20 points per pound of fat plus 4 points per pound of solids-not-fat. No distinction is made as to size of animal except indirectly by distinguishing between first-kidders and older³ animals. Since a 100-pound goat competes on a par with a 200-pound goat in terms of absolute yield it follows that selection must be more severe in the smaller size animals. This tends to bias FCM/W, as concerns an unselected population, making it unduly large for small goats and unduly small for large goats. Just how far this may affect the data of figure 2 (and figure 1) cannot be said.

What is needed is records on an unselected population for the first 8 months of lactation with live weight measured within 31 days after kidding. In the present records variation in the stage of lactation affects not only the daily yield for the 2-day period but affects also the numerical value of b in the regression, $FCM = aW^b$, at least such is true in cows. For example, in Holstein cows, dealing with 8-months FCM and live weight measured at 10 stages of lactation, viz., within the first month (31 days) after calving, within the second month, etc., b is affected as follows:

Month	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
b =	1.01	0.96	0.93	0.94	0.92	0.89	0.91	0.82	0.82	0.83

For the present goat data the average stage of lactation is within the 7th month after kidding and $b = 0.91$ which agrees with the 7th month in the Holstein data. By inference if live weight in the goats had been measured within 31 days after kidding we might expect to find $b = 1.01$.

Finally it may be noted that the average of the 318 records is 5.99 pounds FCM per day per 100 pounds live weight, 60 pounds per 1000 pounds live weight. This is perhaps nearly double what we should find in cows under comparable conditions. Metabolic tempo (metabolism per cell

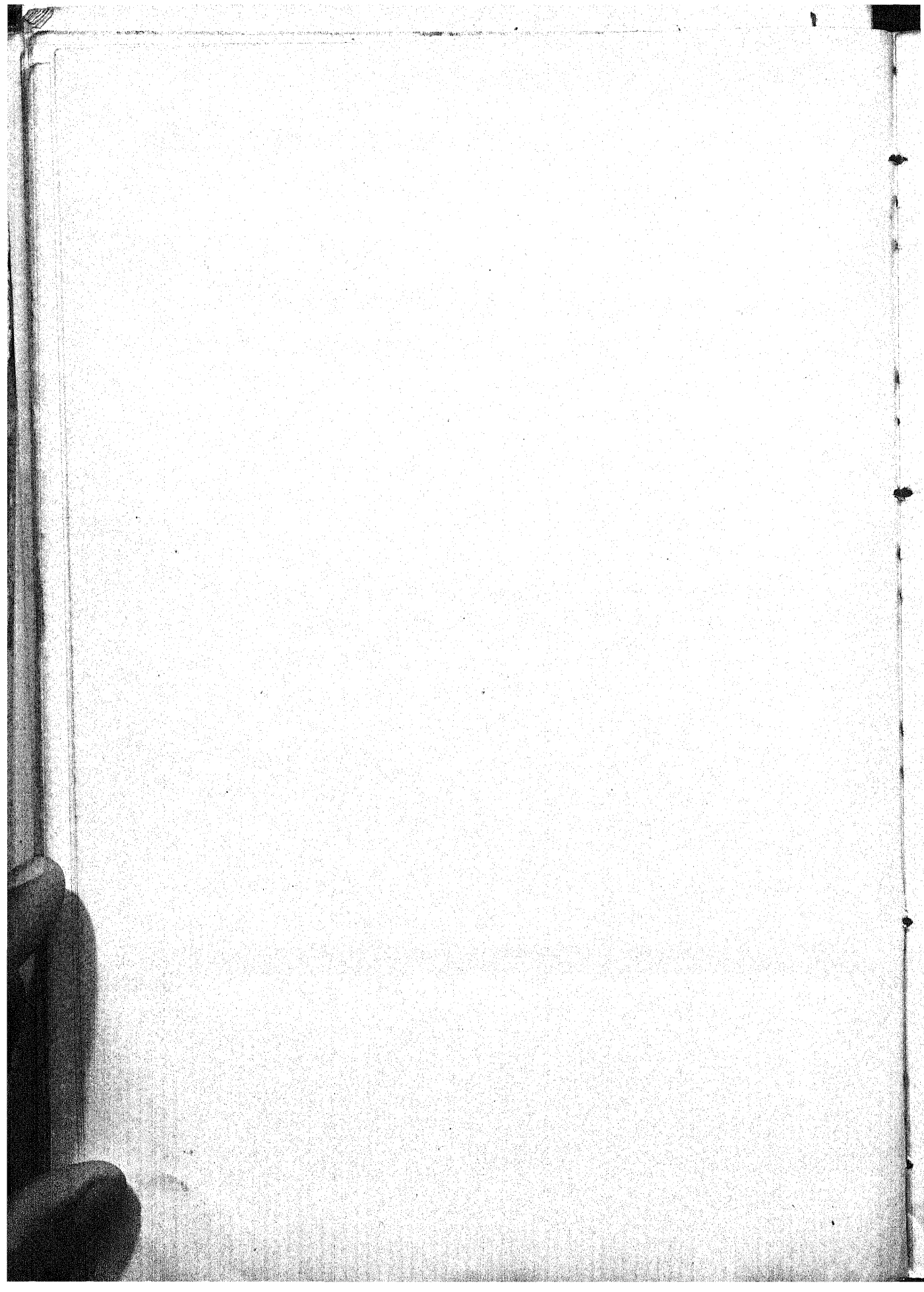
³ In table 2 it will be noted there is no significant difference between age classes with respect to either FCM or FCM/W. For these records age distinctions are wholly without importance, but live weight distinctions are very important.

per unit time) as between goats and cows follows a " $\frac{3}{4}$ power rule" with respect to live weight; but within species metabolic tempo is independent of live weight.

SUMMARY AND CONCLUSIONS

This article deals with 318 records of goats in two-day milking competition at the London Dairy show. The milk-energy yield, FCM, ranges from 1.5 to 17.2 pounds per day. Live weight, W, ranges from 88 to 250 pounds. The correlation between W and FCM is 0.57. In the power equation regression, $FCM = aW^b$, $b = 0.91$.

Attempting to bring the data into line with FCM for the first 8 months of lactation and live weight measured within 31 days after kidding it appears probable that b in the regression $FCM = aW^b$ may be safely taken at unity. That is, milk-energy yield in well-developed milch goats tends to be proportional to live weight.



FURTHER STUDIES ON OXIDATION OF VITAMIN A AND CAROTENE IN MILK FAT

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Our previous study (2) of the effect of storage temperature upon the rates of destruction of vitamin A and carotene in milk fat protected from the light but exposed to the air has indicated that following an induction period, simultaneous progressive destruction of vitamin A and carotene occurs in samples held at 40°, 50°, and 60° C. Since that time additional data have been obtained concerning the changes in vitamin A and carotene contents of the milk fat held at lower temperatures than its melting point; namely, the region within which under favorable conditions the fractional crystallization of glycerides takes place.

We also reported that the reconstitution test is very useful in recognizing the flavor defects of the milk fat and thus the extent of oxidative deterioration. This was evident from the fact that the milk made of pasteurized skimmilk and post-induction period fat developed off flavors, which continued to increase rapidly in their intensities instantaneously after processing, while the one containing fresh fat showed fairly good keeping quality. Subsequently it was observed that the latter milk at the end of 24 hours' storage at 5° C. developed off flavor, although of much lower intensity than the other but nevertheless identified by judges as slightly oily or tallowy and which lowered its score from 23 to 21 points, as compared with the score of zero for the first milk at the end of the same period.

Since the conditions for oxidation appear to be favorable, when fat is redispersed in skimmilk, we can assume that the above technique can be of value in readily detecting changes in the resistance of milk fat to oxidation, whether these changes are brought about during storage, by exposure to light, or any other factor.

For these studies the vitamin A and carotene contents of the fat were determined by the Koehn and Sherman method (3), while in the preparation of milk fat we followed the same procedure as described in the former paper (2).

EXPERIMENTAL

The Effect of Rapid Solidification of Milk Fat Upon the Rates of Destruction of Vitamin A and Carotene

In a study of the relationship between the temperature of storage and the rates of destruction of vitamin A and carotene in the milk fat (2), it was

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pointed out that the samples of fat in the "liquid" state were placed in incubators at constant temperatures. At the end of the holding period at temperatures below the melting point, these samples were found to be well separated into solid crystalline and liquid fractions. It is apparent, therefore, that the system involved was quite different from the one represented by fat stored at higher temperatures.

The additional data concerning this particular experiment are presented in figure 1. They show that at higher temperatures vitamin A and carotene are destroyed at approximately the same rate, whereas at lower temperatures vitamin A is destroyed more rapidly.

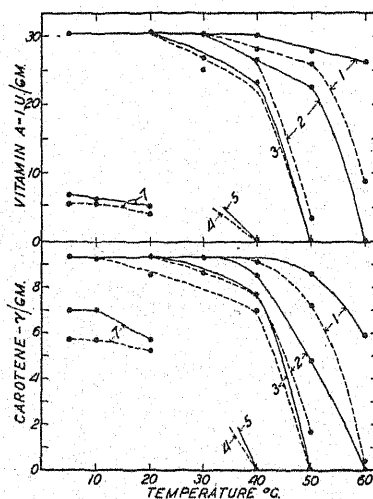


FIG. 1. The relation between the temperature of storage and the rates of destruction of vitamin A and carotene in the fat from (1) milk (solid line) in which lipolysis was checked by pasteurization immediately after excretion by mammary gland, and (2) milk (dotted line) in which lipolysis was accelerated. The fat was exposed to the air but protected from the light. Numbers 1-7 on the graphs indicate months of storage.

There are no significant differences in the rates of destruction between the samples held at 5°, 10°, and 20° C., or between the fats isolated from normal and rancid milks.

In order to learn if the physical state of the fat at the time of storage was a factor governing the rates of destruction of vitamin A and carotene, 25 grams of fat were weighed into half-pint milk bottles, to provide a sufficient surface contact between the fat and outside air. Several of these samples were held first at -14° C. for a period of time to insure a rapid solidification of the fat and then stored at 5° and 20° C. The remaining samples while still in a liquid state were placed at the same temperatures.

The data are presented in table 1. It is apparent that rapid precooling of fat retards the destruction of vitamin A and carotene during the storage at both 5° and 20° C.

TABLE 1

The effect of solidification of milk fat upon its vitamin A and carotene

Fat from normal pasteurized milk

Physical state previous to storage	Stored at temperature	Vitamin A	Carotene
	$^{\circ}\text{C.}$	<i>I.U./gm.</i>	$\gamma/\text{gm.}$
S.	5	21.1	4.3
L.	5	13.6	3.5
S.	20	18.4	4.2
L.	20	11.0	3.8

The samples were analyzed at the end of seven months' storage in the dark. Symbols indicate: S.—solid, and L.—liquid, fats, respectively.

The physical states of these samples at the end of storage were quite different. The samples which were cooled rapidly at -14°C. remained firm, retaining their homogeneous fine texture; the others had a coarse spongy semi-solid texture. Since atmospheric oxygen plays an important part in oxidative deterioration of milk fat protected from the light, it is possible to assume that the destruction of vitamin A and carotene might be traced in part to the rates of diffusion of atmospheric oxygen into the samples.

The keeping quality of different solid fats might vary with their degree of hardness, a factor determining the rate of crystallization.

These results suggest that a functional relationship exists between the

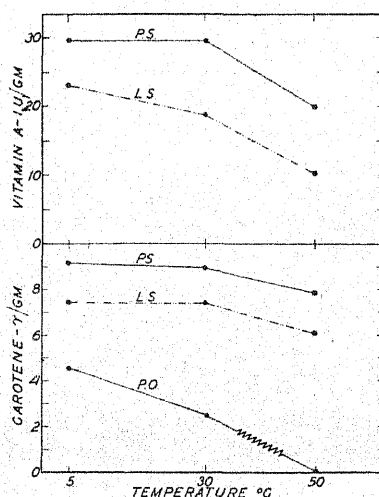


FIG. 2. The vitamin A and carotene contents of samples of milk fat in tightly sealed (S.) or open (O.), plain (P.) and lacquered (L.) tin cans after seven months' storage at indicated temperatures. In plain open tin can (P.O.) the sample was colorless at the end of two and one-half months of storage at 50°C. In the open lacquered cans the samples were all colorless at the end of seven months of storage.

rates of influx of atmospheric oxygen on one hand and the rates of destruction of vitamin A and carotene on the other.

In order to test this possibility, samples of fat in tightly sealed or open tin cans, both lacquered¹ and plain, were placed while still in the liquid state at temperatures of approximately 5°, 30°, and 50° C. These cans were filled to the top. The vitamin A and carotene contents of the tightly sealed samples were determined after storing for 7 months, while carotene only was determined in the other samples.

The data presented in figure 2 show that in the absence of free surface contact with atmospheric oxygen the destruction of vitamin A and carotene proceeds at a much slower rate or not at all, depending upon the temperature of storage and the type of container used.

It seems probable, therefore, that in absence of light, only when fat is exposed to atmospheric oxygen is its physical state a factor governing the rates of destruction of vitamin A and carotene. Subsequently, it was found that the induction period of fat could be considerably prolonged by covering the exposed surface with a sheet of tin foil to exclude the air.

The Destruction of Vitamin A and Carotene Resulting from Reemulsification of Milk Fat in Pasteurized Skimmilk

Preliminary work has been done to show the extent to which vitamin A and carotene of the milk fat is reduced by reemulsification in skimmilk and some of the factors involved in this phenomenon.

In a previous paper (2) it was shown that at the point when the vitamin A content of fat exposed to light generated by a mercury vapor lamp was reduced to a minimum, the concentration of its precursor remained practically unchanged. The data in figure 3 show that at the end of 30 minutes of exposure the vitamin A drops from a value of 33 I.U./gm. to 4.5 I.U./gm. Irradiation for an additional 90 minutes causes no further drop in vitamin A. It did not seem reasonable to us that part of the vitamin A would resist destruction, so it may be assumed that the value of 4.5 I.U./gm. is due to an artifact. That this is so, is supported by the observation that no absorption peak in the 620 mμ region of the spectrum could be detected with the Beckmann spectrophotometer following the Carr-Price reaction, nor could an absorption peak be found in the 326 mμ region with the unsaponifiable fraction dissolved in diethyl ether.

It was found (2) that atmospheric oxygen plays an important part in the photochemical destruction of carotene, while in the case of vitamin A an additional photochemical reaction caused its rapid destruction. Therefore, it seemed of interest to learn if the exposure of fat to light, prior to its reemulsification in pasteurized skimmilk, renders carotene more susceptible to oxidation.

¹ Sanitary enamel.

For this reason the irradiation experiment was repeated following the same procedure as previously described, with the exception that the temperature of fat was maintained at 50° C. and the intensity of the mercury vapor lamp at 19,000 foot candles.² The samples were irradiated for various periods of time up to 120 minutes. At the end of each period they were divided into two parts. One part was immediately analyzed for vitamin A and carotene, while the other one was reemulsified³ in pasteurized skim milk, then held for 24 hours before separation and analysis.

The data are presented in figure 3. Although the carotene content of the samples was not altered by irradiation, its susceptibility to oxidation in the

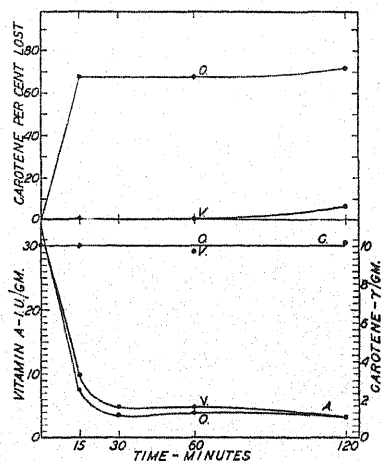


FIG. 3. The effects of irradiation and of reemulsification of irradiated milk fat in pasteurized skim milk upon vitamin A and carotene. The effect of irradiation alone is shown in the upper chart. The lower chart shows the losses in carotene (per cent) due to reemulsification of fat. Symbols on the graphs indicate: C—carotene, A—vitamin A, O. and V.—fat irradiated in open or under vacuum sealed tubes.

presence of skim milk was definitely affected when the fat was irradiated in open tubes. In this case 70 per cent of the carotene was lost due to the reemulsification of fat in pasteurized skim milk, whereas the fat which was irradiated in vacuum sealed tubes was not affected.

Subsequently it was thought worth while to obtain some idea concerning the destruction of vitamin A and carotene produced by irradiation, such as might be encountered under ordinary conditions.

Samples of fat in 18-mm. test tubes filled to the top and left open were irradiated for 10 hours with northern daylight passing through window glass.⁴ (This sample had been stored for 4 months at 5° C. in the dark.)

² Weston light meter.

³ In Club aluminum cream maker.

⁴ The per cent transmission of incident light (Beckmann spectrophotometer) of this glass was as follows: 360 mμ—75.3%; 340 mμ—42.0%; 330 mμ—16.5%; 325 mμ—7.0%; 320 mμ—2.0%; 310 mμ—0%.

TABLE 2
The effect of irradiation with daylight upon vitamin A and carotene of milk fat
 Milk fat

Irradiated with daylight		Reemulsif. in pasteur. skimmilk	Vitamin A	Carotene
<i>Hrs.</i>	<i>In</i>		<i>I.U./gm.</i>	γ / <i>gm.</i>
U.	Before	19.4	9.6
U.	After	11.0	9.2
10	O.T.	Before	6.0	8.3
10	O.T.	After	5.1	1.8
U.	Before	29.5	9.0
U.	After	12.3	7.8
8	P.D.	Before	12.3	7.4

O.T.—open tube, P.D.—Petri dish, U.—unirradiated control.

The light intensity throughout the duration of exposure varied from 200 to 400 foot candles as measured by a Weston light meter. Immediately after, a portion of this fat and one of the control samples were reemulsified separately in pasteurized skimmilk to produce a reconstituted milk containing 5 per cent fat. They were re-separated after holding for 24 hours at 5° C. These samples and the remaining portions were then analyzed for vitamin A and carotene. In another experiment a sample of fat in a covered Petri dish filled to the top was exposed for 8 hours only. (This sample had been stored for 7 months at 5° C. in a tightly sealed plain tin can.)

The data are presented in table 2. These results are in substantial agreement with the preceding observations.

A study of absorption spectra and chromatograms of carotene from irradiated and non-irradiated milk fat samples indicate that no change in the carotene occurred. The irradiation was carried out as previously described in 18-mm. test tubes by northern daylight. This indicates that the effect of irradiation is upon constituents of the fat other than carotene.

Finally the data of table 3 show the effect of irradiation with northern daylight upon vitamin A and carotene of fresh milk fat. To part of the

TABLE 3
The effect of irradiation with daylight upon vitamin A and carotene of fresh milk fat
 Milk fat

Irradiated with daylight		Reemulsif. in pasteur. skimmilk	Vitamin A		Carotene
<i>Hrs.</i>	<i>In</i>		Original	Original and added	
			<i>I.U./gm.</i>	<i>I.U./gm.</i>	γ / <i>gm.</i>
U.	Before	22.1	250.0	5.1
U.	After	19.3	246.0	4.7
8	O.T.	Before	21.7	221.0	5.0
8	O.T.	After	17.0	163.0	3.9

O.T.—open tube, U.—unirradiated control.

sample crystalline vitamin A was added so that it contained 250 I.U. of vitamin A per gram of fat. Both samples of fat in 18-mm. test tubes were irradiated for 8 hours. The light intensity throughout the duration of exposure was less than 200 foot candles.

The results of this experiment were practically the same as the others, except that the losses in vitamin A produced by reemulsification of fresh unexposed fat in pasteurized skimmilk were rather small.

These observations appeared to be of a practical importance since they show that considerable losses in vitamin A, accompanied by the development of oily flavor, occur in the fat exposed to light, thus affecting not only the nutritive value of the product but its palatability as well.

Finally it should be noted that the destruction of vitamin A in the milk fat was accompanied by the development of oily flavor, whereas that of carotene was accompanied by the development of tallowy flavor (1, 2).

The data suggest that a relationship exists between the ability of fat to resist oxidation and the stability of carotene. It is possible that a partial or complete destruction of antioxidant renders fat more susceptible to oxidation, thus indirectly affecting carotene.

CONCLUSIONS

The present study shows that the resistance of both vitamin A and carotene to oxidation by redispersing the fat in pasteurized skimmilk decreases upon exposure of fat to light or after its prolonged storage in the dark.

Milk fat can be stored for several months even at 60° C. without loss of vitamin A or carotene providing the fat is degasified then placed in light-proof containers filled to the top and tightly sealed.

Rapid precooling as compared with slow cooling of milk fat retards the destruction of vitamin A and carotene during its storage at both 5° and 20° C. in open-to-the-air but protected-from-the-light containers.

The data indicate that milk fat containing free fatty acids shows more rapid loss of vitamin A and carotene during its storage at 40°-50° C. in open-to-the-air but protected-from-the-light containers.

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ZINC IN COWS' MILK*

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INTRODUCTION

References in the literature to the zinc content of cows' milk are rather infrequent. Several investigators report its presence in the spectrographic analysis of milk ash (3, 6, 7, 11, and 12), but only three report quantitative determination of the amount present (2, 4, and 8). Information on the influence of the amount of zinc in the feed on the zinc content of milk is practically negligible. Wright and Papish (11) in 1929 found more than the usual amount of zinc in milk obtained from cows pasturing in the neighborhood of a zinc smelter. Broek and Wolff (5) in 1935 found no marked variations in this element that could be attributed to feeding practice.

As part of a comprehensive project on the minerals of cows' milk, the effect of feeding zinc oxide on the zinc content of milk was investigated at this station during the past winter (1942-43).

EXPERIMENTAL

The procedure was similar to that followed in earlier work on manganese (1). Eight cows were divided into two groups of four each, with an Ayrshire, a Guernsey, a Holstein, and a milking Shorthorn in each group. The breed pairs were matched as closely as possible with regard to stage of lactation, none of the cows being beyond the 10th week in lactation when the work was begun. One group received the supplement during November, December and January; the other group received it during February, March and April. The amount of zinc oxide fed was ten grams daily mixed with the grain allowance. No ill effects were evident as a result of feeding this amount of zinc. Except for the feeding of the supplement the rations and management of the two groups were in all respects identical.

Composite two-day milk samples of one liter each were taken from each cow once a month. Zinc was determined in triplicate 200 ml. portions of each sample by the turbidity method of Bodansky (4) as quoted by Scott (10). As some slight modifications were introduced, the detailed procedure is given below.

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¹ Acknowledgment is made to Vernon C. Cole, who did the analytical work under the author's direction.

Method for Determination of Zinc in Milk

Solutions required: *Standard zinc solution.* Dissolve 0.1 gram of zinc (C.P.) in 10 ml. of HCl (sp. gr. 1.20); Dilute to one liter. 1 ml. = 0.0001 gram of zinc.

Citric acid solution—50%

Sulphuric acid—1 in 3.

Ammonium thiocyanate solution—2%

Hydrochloric acid—1 in 5.

Potassium ferrocyanide solution—2%

Procedure. Measure out 200 ml. of milk into a glazed silica evaporating dish and evaporate to crispness on a steam bath. Ash in an electric muffle furnace at just below perceptible redness; the dishes are placed in the cold furnace and the temperature is raised gradually to avoid frothing and spattering. The ash should be white or with not more than a tinge of gray. Cool and dissolve the ash with 25 ml. conc. HCl and 10 ml. conc. HNO_3 ; boil moderately on a hot plate for half an hour. Add 10 ml. of H_2SO_4 (1 in 3) and evaporate to dryness on a steam bath. Dissolve the residue in water and adjust the acidity to contain about 5 per cent H_2SO_4 (probable volume 150 cc.) and precipitate the heavy metals by bubbling H_2S through the liquid for an hour.

Filter off the heavy metals and boil the filtrate on a hot plate to remove H_2S ; cool, add two drops of methyl red, neutralize with NH_4OH and add 10 ml. of a 50 per cent citric acid solution. Heat to boiling on a hot plate (watch out for bumping at this point) and if no calcium citrate separates add small quantities of calcium carbonate at a time until a precipitate of about one gram of calcium citrate is formed. Remove from heat and while still very hot pass a stream of H_2S through the solution until cool and for four hours thereafter. Filter through a small paper and wash with a 2 per cent solution of ammonium thiocyanate. Dissolve the precipitate in 25 ml. of HCl (1 in 5) heated almost to boiling, wash the paper with water, cool the filtrate and make up to 50 ml.

Place a 25-ml. aliquot of the filtrate in a 50-ml. Nessler tube and hold until a series of standards of appropriate range has been prepared by measuring portions of the standard zinc solution into other 50-ml. Nessler tubes. Dilute the standard and unknown solutions to about 40 ml. and add 3 ml. of HCl (sp. gr. 1.20) to the standards, and 2 ml. of 2 per cent potassium ferrocyanide solution to all the tubes. Dilute the contents of each tube to the mark and mix thoroughly. After two or three minutes compare the turbidity of standards and unknown in a suitable comparator rack with a white tile base and illuminated by fluorescent light. Calculate the percentage of zinc from the quantity of sample taken and the standard solution similar in turbidity to that of the sample. Standards used in this work were 2, 3, 4, 5, 6, 7, and 8 ml. of the standard zinc solution per 50 ml. of final volume. The method is sufficiently sensitive to permit matching to one-half ml. of the standard (0.00005 gram of Zn).

TABLE 1
Effect on zinc content of the milk of feeding cows zinc oxide (winter of 1942-43). Milligrams of zinc per liter of milk

Month	Cows receiving supplemental Zn				Cows on control ration					
	1st half of the season				2nd half of the season					
	A291	G626	H521	S38	Average all four	A320	G632*	H539	S62	Average all four
November	3.8	7.2	5.7	5.7	5.6	3.4	5.3	3.4	1.9	3.5
December	6.3	7.2	4.3	6.7	6.1	5.8	3.9	4.3	5.3	4.8
January	4.3	6.7	4.3	3.8	4.8	3.4	4.3	3.4	2.8	3.5
Average 1st half	5.5	3.9
2nd half of the season										
February	A320	G605	H539	S62	Average all four	A291	G626	H521	S38	Average all four
March	6.3	3.4	3.8	4.8	4.6	4.3	3.4	1.9	5.3	3.7
April	4.8	3.4	5.3	4.8	4.6	2.9	3.4	2.4	3.8	3.1
Average 2nd half	4.3	3.8	3.8	6.7	4.7	3.4	4.8	3.4	4.3	4.0
Average entire season	4.6	3.6
					5.1					3.8

* Through a misunderstanding this cow was sold soon after the December samples were taken—G605 was substituted for her so that the values for January to April inclusive are for 605.

NOTE.—The initial letter prefixed to each cow's number indicates the breed.

RESULTS

The values obtained are set forth in table 1. The difference of 1.3 mgs. per liter in the zinc content of the milks from the two groups is statistically significant. A smaller difference was noted in the second half of the season than in the first, but this also is significant. It is believed that the difference between the groups in the second half would have been greater if Cow G632 had been retained in the herd. Rather consistently low values for zinc were obtained from analysis of the milk samples from the substitute Cow G605. Her milk also had a consistently low total ash content for a Guernsey (an average of 0.68% for the four months she was in the group as compared with 0.74% for her breed mate, G626, for the same four months). Also her milk looked abnormal, suggesting the high color of colostrum, although not above 4.7% fat at any time during the whole four months and running as low as 4% in March and April.

It is definitely suspected, therefore, that here was an abnormal milk, and if in consequence it be eliminated, the difference in zinc content of the milks in the second half of the trial is raised to about the same magnitude as it was in the first half, *i.e.*, approximately $1\frac{1}{2}$ mgms. per liter more zinc in the milk when the cows were fed zinc oxide than when they were not.

In agreement with the work of Birekner (2) considerable individual variation is noted in the amounts of zinc from the different cows, but it should be observed that out of twenty-four comparisons, zinc was higher in the milk from the control group in only two instances and in one of these two the apparently abnormal milk already referred to was involved.

The amounts of zinc in the control samples are in good agreement with those reported by Birekner (2) and by Sato and Murata (9).

SUMMARY

Zinc oxide was fed as a supplement (10 grams per cow daily) to the ration of eight cows for a period of three months by the double reversal method. The milks from the cows were analyzed for their zinc content and it was found that feeding the zinc supplement had consistently raised the level of that element in the milk, the average being 5.1 mgms. of zinc per liter of milk as contrasted with an average of 3.9 mgms. when the cows were on a control ration.

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THE DIGESTIBILITY OF KOREAN LESPEDEZA HAY AND GROUND KOREAN LESPEDEZA SEED FOR DAIRY HEIFERS*

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Korean lespedeza is playing an increasingly large part in the feeding program of farm animals. It is widely used as a fine mid-summer pasture crop and as hay; and the abundant yield of seed can be utilized as a high protein concentrate for livestock feeding. Although the high feeding value of Korean lespedeza has been shown often in comparative feeding trials, few reports have appeared concerning the digestibility of the hay fed alone. The investigation reported herein was therefore instituted for the purpose of determining the digestibility of different types of Korean lespedeza hays and the digestibility of Korean lespedeza seed fed with different hays.

MATERIALS AND METHODS

Three lots of Korean lespedeza hay were used. Each was grown on a different farm near Columbia, Missouri. The fertility of the soil on which each was grown was only fair. The fields had been clipped while the lespedeza was small so that the hays were practically pure lespedeza. One lot, secured in 1941, was cut about two weeks previous to the start of blooming. In 1942 one lot was made just as blossoms were starting to appear and a second lot was made when blooming was over and the seeds were mostly in the dough stage. The alfalfa hay was third-cutting Missouri-grown hay of good quality.

The Korean lespedeza seed was cleaned seed secured on the open market.

Holstein-Friesian heifers 18 to 20 months old were used as experimental animals. The digestibility of each ration was determined with four heifers. Collection periods were of 10 days duration following a 10-day preliminary feeding period in which a constant daily amount (16 pounds) of the ration was fed.

The hays were chopped in a hammermill using a 1-inch screen. The seed was twice ground through a $\frac{3}{8}$ -inch screen of the hammermill. The entire amount of hay or hay and seed to be fed was thoroughly mixed and sacked into daily rations previous to starting the trials. At the time of sacking the feeds were sampled for moisture determination. The composition of the feeds was determined from composite samples representing the entire lot. The lespedeza hay and seed rations were composed of 1 part seed and 3 parts hay. Four parts of alfalfa hay were used per part of seed.

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TABLE 1
Average composition of feeds used in digestion trials and of feces, dry basis

	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract	Lignin	Cellulose	Other carbohydrates
	%	%	%	%	%	%	%	%
(1) 1941 lespedeza hay (early)	12.42	3.16	5.33	32.17	46.92	15.93	30.96	32.20
(2) 1942 lespedeza hay (intermed.)	11.84	2.37	5.48	34.09	46.22	19.97	34.50	25.84
(3) 1942 lespedeza hay (late)	13.28	2.76	5.45	37.02	41.49	23.14	33.13	22.24
(4) 1942 alfalfa hay	16.94	1.95	10.07	32.82	38.21	11.42	33.86	25.75
(5) 1941 lespedeza seed	36.39	9.95	5.80	10.28	37.58	5.67	13.29	28.89
(6) 1942 lespedeza seed	39.42	5.67	6.21	13.88	34.83	10.33	15.39	22.98
(7) Lespedeza seed + early hay	18.58	4.91	5.44	26.55	44.52	13.29	26.43	31.36
(8) Lespedeza seed + late hay	20.02	3.51	5.65	31.06	39.76	19.83	28.50	22.49
(9) Lespedeza seed + alfalfa	21.61	2.73	9.27	28.88	37.51	11.20	30.02	25.17
Feces from (1)	14.40	5.33	10.50	35.08	34.68	31.08	27.13	11.55
Feces from (2)	13.28	4.30	9.90	32.67	39.85	34.88	27.46	10.18
Feces from (3)	13.86	4.39	9.38	32.78	39.59	36.58	25.58	10.21
Feces from (4)	14.14	4.49	14.32	37.30	29.76	25.66	29.06	12.33
Feces from (7)	15.63	6.62	15.83	30.33	31.59	28.66	25.15	8.11
Feces from (8)	15.44	5.33	9.67	32.74	36.82	34.01	26.03	9.52
Feces from (9)	15.00	5.42	13.14	34.21	32.23	27.51	28.81	10.12

The feces were collected by an automatic separator patterned after an apparatus described by Ritzman and Benedict (7). A 3 per cent aliquot sample was taken from each day's fecal output from each heifer. The daily samples were composited, moistened with 2 per cent hydrochloric acid, and stored in a refrigerator at 5° C. until the completion of the collection period. The feces were then thoroughly mixed, dried with stirring in a Freas electric drying oven at 50° C., allowed to reach weight equilibrium in the laboratory, ground in a Wiley mill, and sampled for analysis.

Analyses were made of the feeds and feces by methods prescribed by the A.O.A.C. (2) for nitrogen, ether extract, crude fiber, ash, and nitrogen-free extract. Moisture was determined by drying in a drying oven at 105° C. until weighings at 2-hour intervals showed no further loss. Lignin, cellulose, and other carbohydrates were determined according to the method of Crampton and Maynard (3) except that the ether-extracted sample was moistened and autoclaved previous to digestion with 0.5 per cent pepsin in N/10 hydrochloric acid.

Digestion coefficients, total digestible nutrients, and nutritive ratios have been determined in the usual manner.

RESULTS AND DISCUSSION

The composition of the feeds and rations fed and the average composition of the feces from each ration are listed in table 1 on the dry basis. These show that lespedeza hay does not decrease in protein content with advancing maturity but that lignification is quite marked as the plant matures. The high protein content of lespedeza seed is demonstrated. The seed also has a sizeable portion of fiber due to the hulls which normally adhere tightly to the seed.

The average digestion coefficients, total digestible nutrients, and nutritive ratios which were calculated for the different rations and feeds are presented in table 2.

The effect of advanced maturity upon the nutritive value of Korean lespedeza hay is clearly shown in table 2. With increased maturity there was a decrease in digestibility of practically every nutrient division. While lespedeza hay cut well in advance of bloom was very acceptable as a source of total digestible nutrients, late cut hay was markedly inferior. It is also worthy of note that the nutritive ratio of the good lespedeza used here was much wider than the 1:4.7 estimated by Morrison (6). This is due to the fact that the protein of Korean lespedeza hay is not digested as thoroughly as that from many other legumes. The high lignin content of lespedeza leaves has been proposed by Swanson and Herman (9) as an explanation of the poorer protein digestion from lespedeza hay.

The data in table 2 also present further evidence of the importance of the lignin content of the feed to its utilization. There is a remarkably close

TABLE 2
Digestion coefficients, total digestible nutrients, and nutritive ratios of Korean Lespedeza hay and seed

	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Lignin	Cellulose	Other carbohydrates	Total dry matter	Total dig. nutr. (dry basis)	Nutritive ratio 1:
	%	%	%	%	%	%	%	%	%	
1941 lespedeza hay (early)	49.17	29.15	54.21	68.90	17.99	63.18	84.91	57.95	57.95	8.48
1942 lespedeza hay (intermed.)	42.51	7.06	50.82	55.78	10.40	59.16	79.79	48.70	48.51	8.64
1942 lespedeza hay (late)	41.03	10.18	49.92	46.03	10.60	56.34	74.01	43.44	43.66	7.01
1942 alfalfa hay	65.10	5.00	52.42	67.47	3.99	64.09	79.96	58.17	54.23	3.92
Lespedeza seed + early hay	63.51	41.49	50.41	69.26	6.62	58.69	88.76	56.64	60.59	4.13
Lespedeza seed + late hay	61.70	24.59	47.57	53.99	14.80	54.58	78.87	50.29	50.54	3.09
Lespedeza seed + alfalfa hay	72.52	21.34	53.11	65.96	3.00	61.99	84.06	60.39	57.06	2.64
Lespedeza seed (- early hay)	77.66	52.85	20.82	70.56	0	27.39	94.94	52.86	68.76	1.43
Lespedeza seed (- late hay)	81.74	44.86	30.28	81.29	41.90	43.52	90.13	70.01	70.45	1.19
Lespedeza seed (- alfalfa)	84.66	42.66	59.33	59.67	1.85	44.45	97.84	68.87	67.83	1.03
Average of all lespedeza seed	81.35	46.79	36.81	70.51	14.58	38.45	94.30	63.91	69.01	1.22

relationship between the lignin content and the digestibility of the three lots of lespedeza hay. The lignin-digestibility relationship does not seem the same for the alfalfa hay as for the lespedeza hay, however. This may be due to the fact that the heifers did not ruminate on the alfalfa rations, presumably because it was ground too fine, but did ruminate on all of the lespedeza hay rations. This lack of rumination may have slightly depressed the digestibility of the alfalfa hay. Ritzman and Benedict (8) found that ground alfalfa hay was less digestible than unground hay. It is also probable that the type and manner of deposition of lignin is so different in alfalfa and lespedeza that their relative amounts are not an adequate measure of their effect upon digestibility of the two kinds of hay.

A few seeds from the late-cut lespedeza hay came through the heifers whole. This loss of nutrients may have accounted for part of the low digestibility of the late-cut hay. The undigested seeds were not in great numbers, however, and it is believed that their effect upon lowering the digestibility of the hay was of minor importance compared to the effect of the high degree of lignification of the plant.

In drawing conclusions about the effect of maturity upon the digestibility of Korean lespedeza hay from these data, one must consider that the three lots of hay came from three different farms and were grown in two different seasons. Hays of different maturity from the same field grown in the same season may show slightly different variations than are shown in table 2.

The digestibility of Korean lespedeza seed was determined with three lots of hay, early-cut lespedeza hay, late-cut lespedeza hay, and alfalfa hay. The average results of the trials with each hay and the average of all of the lespedeza seed digestibility calculations are presented in table 2. It is shown there that the crude protein and the non-fibrous carbohydrate of lespedeza seed are quite highly digestible. Great variation was found in the digestibility of the fibrous portion of the seed. The seed was moderately high in total digestible nutrients, 69.01 per cent on a dry basis, and furnished a large amount of digestible protein, the average nutritive ratio being 1:1.22. The differences in digestibility of the seed fed with the different hays were not of noteworthy importance when it is considered that two different lots of seed were used. The 1941 seed was higher in ether extract and its ether extract was digested better than that of the 1942 seed.

Many analyses of lespedeza seed at the Missouri station have shown that it contains approximately 35 to 40 per cent total crude protein. This would be equivalent to about 28.5 to 32.5 per cent digestible crude protein. The ground lespedeza seed was eaten with relish by every one of the eight heifers used. It did not appear to exert any abnormal effect upon the animals. Experiments by Herman and Ragsdale (4), Irwin and Kempster (5), and the Missouri Department of Animal Husbandry (1) have shown that Korean lespedeza seed serves satisfactorily in rations of dairy cattle, beef cattle,

poultry, and sheep. It should therefore be considered as a very nutritious high protein concentrate which well deserves the attention of farmers seeking to increase their supply of home-grown high protein feeds.

SUMMARY

Digestion coefficients were determined on Korean lespedeza hay cut well in advance of bloom, just before bloom, and after bloom. The digestibility of practically all nutrients was decreased with advanced maturity and the late-cut hay was a very poor source of total digestible nutrients. The lignin content of the hays increased with maturity and as the lignin increased digestibility was decreased.

Digestion coefficients were calculated for ground Korean lespedeza seed fed with two types of lespedeza hay and with alfalfa hay. The lespedeza seed crude protein averaged 81.35 per cent digestible which makes the seed contain approximately 28.5 to 32.5 per cent digestible crude protein. The seed contained an average of 69.01 per cent total digestible nutrients and had a nutritive ratio of 1:1.22.

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CITRUS MOLASSES—A NEW FEED*

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Citrus molasses was produced in Florida on a commercial scale during the canning season of 1941-1942 with a production of 2500 tons. During 1942-1943 this was increased to about 4700 tons. Approximately one-half of domestic blackstrap molasses was unavailable for feeding purposes due to its retention for manufacture of war materials. Hence citrus molasses filled a need in the manufacture of mixed feeds. The supply of citrus molasses has been insufficient to meet the demand of the mixed feed industry.

RECOVERY OF CITRUS MOLASSES

From a survey made by the Department of Agricultural Economics of the Florida station it was determined that 67.5 per cent of bulk citrus fruits remains as peel, rag, and seed, from the canning of hearts, juice, and citrus concentrate. This residue or fresh pulp contains about 85 per cent of moisture mostly as bound water, or water of constitution.

Addition of calcium salts to the fresh pulp liberates the bound water. One-half of the water then is removed by pressure. The pulp is dehydrated into dried citrus pulp. The press juice contains about 5.4 per cent solids—mainly sugars. Evaporation of this material under partial vacuum to about one-thirteenth of the original volume results in a light colored sweet viscous syrup known to the feed industry as "citrus molasses." A glucoside—naringin—which imparts a characteristic flavor to citrus peel, also is concentrated in the process, so that its flavor is accentuated in the molasses.

COMPOSITION OF CITRUS MOLASSES

Citrus molasses is not wholly standardized as to composition perhaps due to changes in the press juice from citrus fruits as the season advances. The producers concentrate it to a reading of 75 degrees Brix. The resulting product varies slightly in total solids content, and in proportions of the different constituents.

Four analyses of citrus molasses are available, 3 from the 1941-1942 crop, and one (Sample No. 4) determined in the Nutrition Laboratory from the 1942-1943 crop. These analyses are given in table 1.

The specific gravity of citrus molasses (Sample No. 4 was 1.376) and a pH value of 4.65 was observed. Analyses showed 0.94 per cent of calcium citrate [$\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)$] present as fine white flakes which settled out on

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TABLE 1
Analyses of citrus molasses

Sample No.	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Reducing sugars	Non-reducing sugars	Weight per gallon
	%	%	%	%	%	%	%	%	lbs.
1	66.8	5.00	0.15	None	57.97	3.57	20.92	19.87	11.07
2	71.2	3.86	0.24	0.03	61.76	5.31	27.74	15.28	11.61
3	75.2	3.75	0.23	None	65.95	5.24	32.00	18.70
4	66.5	3.39	None	None	59.64	3.47	11.17

standing. The major constituents of this molasses are reducing and non-reducing sugars, the proportions of which vary. No fiber is present. The amount of ether extract depends upon the mechanical separation, or the evaporation of citrus oils.

No digestion trials have been conducted using citrus molasses. Digestion trials on similar products included 4 with beet molasses and 26 with cane or blackstrap molasses, cited by F. B. Morrison in "Feeds and Feeding," 20th edition. The weighted average of these digestion coefficients—35 per cent for crude protein and 90.1 per cent for nitrogen-free extract—applied to the average crude protein and nitrogen-free extract from analyses of citrus molasses in table 1, gave an estimated value of 1.4 per cent digestible crude protein and 56.7 per cent of total digestible nutrients, with 69.9 per cent of dry matter in the molasses.

PALATABILITY OF CITRUS MOLASSES

Since the flavor of naringin predominates in citrus molasses, the palatability of the product for dairy cows was questioned. Two brief conclusive palatability trials were conducted with the Jersey cows in the Experiment Station dairy herd using different levels of molasses. Since a 9 per cent level of blackstrap (sugarcane) molasses is the amount present in many commercially mixed dairy feeds five per cent of citrus molasses was incorporated into home-mixed concentrates. Twenty-five cows were offered 2 pounds each of the molasses-concentrates after consuming their regular afternoon allowance of feed. All of the feed was consumed without hesitation. On the second day, 10 pounds of citrus molasses were incorporated with 90 pounds of concentrates; offered in the same manner, and consumed completely by the 25 animals.

Thirty-four Jersey and Guernsey cows were offered straight citrus molasses from one to three times while in the stanchions. Twenty-six animals refused it on first offering, 17 on the second, and 8 on the third offering. Eleven cows ate all of the citrus molasses; 7 took part; 5 tasted it, and three others ate all when mixed feed was sprinkled beside it. When offered first, the majority of cows did not care for the pure product, the proportion decreasing on later offerings. It is believed that cows would learn to take this feed, but it appears undesirable for feeding separately.

EFFECT ON MILK FLAVOR

Does citrus molasses affect the flavor of milk? To answer this question individual milk samples were obtained from four Jersey cows after being in dry lot 10 hours without feed. On other days, citrus molasses was added as 10 per cent of the mixed concentrates, fed 2 hours prior to milking time, and additional milk samples obtained. Samples from the complete milking were collected in milk bottles, cooled immediately in ice water and held at 35° F.

TABLE 2

*Effect of citrus molasses on the flavor of milk, as scored by 3 judges**

	Cow No.				Average
	664	655	708	737	
<i>Cows received no feed 10 hours prior to milking; 2 trials</i>					
Average score	38.1	37.7	38.7	38.7	38.3
<i>Cows received molasses-concentrate feed 2 hours prior to milking; 4 trials</i>					
Average score	35.1	38.1	37.2	38.0	37.1

* The samples were scored according to the American Dairy Science Association score card, which allows 45 points for flavor.

for 15 hours. The samples then were pasteurized at 143° F. for 30 minutes; cooled to 60° F., and scored for flavor by 3 experienced judges.

The average flavor score of milk obtained when no feed was consumed by the cows 10 hours before milking, was only 1.2 points higher than when mixed concentrates containing 10 per cent of citrus molasses were fed 2 hours before milking. At least part of this difference in score can be attributed to the concentrate mixture itself. Also, the milk from cow No. 664 increased in saltiness during the course of the experiment. While some feed flavors were noted, they were neither intense nor objectionable. It is believed that if this product was fed at milking time, no noticeable effect on flavor of milk would result.

CITRUS MOLASSES IN GRASS SILAGE

Blackstrap molasses is used in ensiling grasses and legumes. In its stead, a trial was conducted using Napier grass (*Pennisetum purpureum* Schumach), ensiled without molasses, 2 levels of citrus molasses with Napier grass and one level with pigeon peas (*Cajanus indicus* Spreng.). The levels of citrus molasses used with the forage are shown in table 3.

The relative palatability of these four silages was observed with nine Jersey heifers, based upon the number of animals eating each silage, and the heifers showed a decided preference for the molasses-Napier over the

TABLE 3

Effect of ensiling Napier grass and pigeon peas with citrus molasses, upon its palatability to Jersey heifers

Forage	Proportion of citrus molasses added	Aroma of silage	pH	Preference of heifers
	%			
Napier grass	0	Fair to good	4.08	3rd
“ “	2	Excellent	3.86	1st
“ “	4	Excellent	3.95	2nd
Pigeon peas	4	Good	5.08	4th

plain Napier silage. Although the pigeon pea silage rated fourth in palatability, it ensiled well with the added citrus molasses, and had a desirable aroma.

SUMMARY AND CONCLUSIONS

Citrus molasses is replacing cane molasses in part of the mixed dairy feeds. Over 4700 tons were obtained in the second year of operation, it being recovered along with dried citrus pulp as by-products of the citrus canning industry. Reducing and non-reducing sugars constitute two-thirds of the dry matter. No fiber, little fat, 4 per cent of crude protein, and over 0.9 per cent of calcium citrate were present. Values of 1.4 per cent of digestible crude protein and 56.7 per cent of total digestible nutrients were estimated by applying the digestion coefficients for beet and cane molasses.

Dairy cows found citrus molasses highly palatable mixed in concentrates at 5 and 10 per cent levels, but much less so when first offered separately. A slight feed flavor was imparted to milk when fed 2 hours before milking time. The flavor was neither intense nor objectionable.

Additions of citrus molasses to a non-saccharin grass (Napier) at 2 and 4 per cent levels improved aroma and palatability of the silage. Pigeon peas ensiled satisfactorily with a 4 per cent addition of citrus molasses.

ACKNOWLEDGMENTS

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THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY CONCENTRATE ON SEVERAL PROPERTIES OF MILK.

I. FAT, TOTAL SOLIDS AND ASH CONTENT*

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Cottonseed meal has been one of the most commonly used protein supplements for dairy cows. In the southern states where this meal is the principal locally-produced concentrate feed, it is usually the cheapest source not only of protein but also of total digestible nutrients available to the dairyman. Therefore, it is often economical to feed cottonseed meal as the only concentrate to dairy cows.

Research work at various stations (5, 7, 9, 10, 13, 14, 16) indicate that cottonseed meal is a satisfactory concentrate for dairy cows when it is supplemented with adequate amounts of vitamin A and calcium.

It is recognized that many factors may cause variations in the composition of milk. The results of numerous experiments indicate that the fat, the most variable constituent of milk, cannot be altered permanently to any marked degree when the cow receives what is considered as an adequate ration, but many feeds of high oil content, including cottonseed meal, have been shown to cause at least a temporary increase in the fat percentage (1, 2). In contrast prickly pear and certain oils, such as cod liver oil, have been found to decrease the fat percentage (11, 21). Furthermore, it is extremely difficult to relate directly the variations in the solids-not-fat and the ash of the milk to the feed of the cow. Taylor and Husband (20) and other workers have shown that feed can alter the composition of milk indirectly through an effect on the milk production. The percentage of total solids, of fat and of ash usually is found to vary inversely and the percentage of lactose directly with the volume of milk yielded. Recently Riddet *et al.* (18) have confirmed observations that subnormal feeding results in a decrease of the percentage of solids-not-fat in milk along with a reduction in volume produced.

Since general economic and nutritional conditions in the South indicate the need of conserving and utilizing all the constituents of milk for human consumption, this study was concerned primarily with the effect of cottonseed meal on the milk solids collectively as well as individually.

EXPERIMENTAL

The effects of feeding cottonseed meal as the only concentrate to dairy cows were studied by comparing the properties of the milk produced by a

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group of cows receiving cottonseed meal (plus 2 per cent bone meal and 1 per cent salt) with the properties of milk produced by a similar group receiving a concentrate mixture of 400 pounds corn gluten meal, 200 pounds wheat bran, 200 pounds ground corn, 200 pounds ground oats (plus 2 per cent bone meal and 1 per cent salt). The roughage received was the same for the two groups, either corn and soybean silage or pasture.

The cows used in this experiment were carefully selected from the Holstein herd so that each cow on the cottonseed meal ration was paired with a comparable cow on the control ration. An attempt was made to maintain from eight to ten cows in each group; however, at times the number dropped to five cows per group.

A comparison of the milk from the two groups as to total solids, fat and ash content (4) over a period of sixteen months is presented in figure 1.

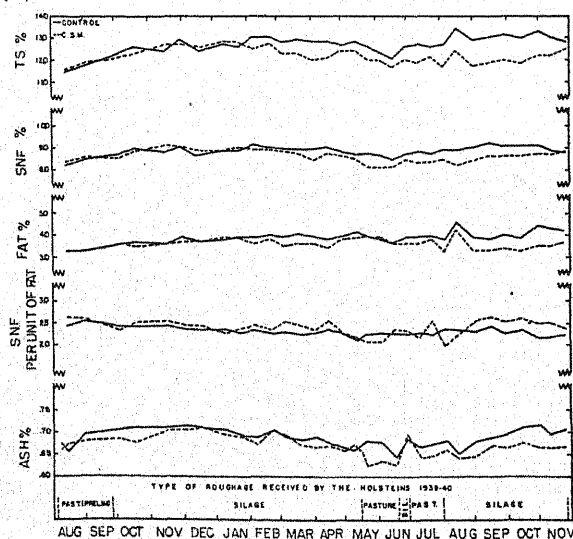


FIG. 1. The total solids, solids-not-fat, fat, ratio of fat to solids-not-fat and ash in the milk produced by a group of cows receiving cottonseed meal as the only concentrate compared with the same constituents in the milk produced by a similar group receiving a normal concentrate mixture.

During the first four months the groups were receiving their respective rations, no differences were evident in the fat and total solids content of the milks. After this time, the milk produced by the cows receiving the cottonseed meal began to have a consistently lower fat and total solids content than the milk from the control group. An exception to this difference in fat content occurred for a brief time when the cows were released on pasture in May.

The solids-not-fat content of the milk from the two groups follows the same trend as the total solids and fat. The ratio of the solids-not-fat to fat

indicates that there was a tendency for the fat to be lower in proportion to the solids-not-fat in the milk from the cottonseed meal group than in that from the control group. This tendency did not hold true during the period the roughage was being interchanged with silage and pasture.

The ash content of the milk from the two groups of cows showed no significant differences until the cows were turned on pasture. This change in roughage was accompanied by a drop in the ash of the milk from the cows receiving the cottonseed meal. The ash increased when the cows were returned to silage, but dropped again when returned to pasture and then remained low even though the cows were once more fed silage.

DISCUSSION

It may be significant that four months on the rations elapsed before any differences in total solids and fat became evident. The differences in the composition of the milk from the two groups showed no apparent correlation with the quantity of milk produced (unpublished data) except for the higher fat and lower production from the cottonseed meal group at the time the cows were first turned on pasture in May. At this stage, several individuals of the cottonseed meal group underwent a temporary physiological upset characterized by anorexia, decreased milk production and abortions. At the same time, the production of the control group increased and the general health of the animals remained good. Under these conditions variations in the composition of the milk were to be expected. The question as to whether the effects on the cows in the test were related to the cottonseed meal ration or to some other factor or factors coincidental with the progress of the experiment remains unanswered. There was no definite indication that the cows were suffering at any time from either a vitamin A or a calcium deficiency.

After the differences in composition of the milk from the two groups had become established, it was planned to reverse the groups, but so many cows had to be removed from the cottonseed meal ration that the group became too small to continue. Another paired series, using Jerseys, was placed on a similar regime. After a period of about four months, the same differences in fat and total solids observed in the milk of the Holstein groups became evident in the Jerseys; but since still more difficulty was experienced in maintaining the organization of these groups, the results cannot be accepted as definite confirmation of the data reported for the Holsteins.

Hills *et al.* (12) and Perkins (17) concluded from their investigations that the level of protein feeding does not affect the composition of milk. On the contrary, Stewart and Tocher (19) observed a decrease in percentage of solids-not-fat from feeding a high protein ration, and Keith *et al.* (15) reported a lower fat and specific gravity in the milk produced on a ration of cottonseed meal and prairie hay than in the milk produced on a standard

herd ration. Although there were other factors involved in the experiment reported in this article, the results indicate that the level of protein feeding might affect the composition of the milk.

The differences in ash content of the milk from the two groups tended to follow the same trends as the differences in solids-not-fat. Becker *et al.* (6) found that calcium and phosphorus of milk remained normal even though the cows were suffering from a severe deficiency of these elements. This is in accord with the general belief that feed does not directly affect the ash content of milk (8). On the other hand, it has been shown that certain elements of the ash can be increased by feed (3). Interesting results might have been obtained from a study of the elemental composition of the ash of the milk from the two groups.

SUMMARY

The percentage of fat, total solids and ash of the milk produced by a group of Holstein cows receiving cottonseed meal as the only concentrate was compared with that of a similar group receiving a mixture of corn gluten meal, wheat bran, ground corn and oats. The roughage was the same for the two groups. The data collected covered a feeding period of sixteen consecutive months.

Four months after placing the animals on their respective experimental rations the milk produced by the group receiving the cottonseed meal ration had a lower percentage of total solids, fat and solids-not-fat than that from the control group. Later the ash content likewise became lower.

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THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY CONCENTRATE ON SEVERAL PROPERTIES OF MILK.

II. NITROGEN DISTRIBUTION*

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Investigations (2, 5, 9, 11) have shown that feeding a high level of protein increases the non-protein nitrogen content of the milk. Although this is generally accepted to be the only direct effect of feed on the nitrogen composition of milk, some investigators (3, 10) have observed a slight variation in other nitrogen fractions accompanying changes in the level of protein feeding.

In a preceding report (4) the total solids, the fat and the ash content of the milk produced by a group of cows receiving cottonseed meal as the only concentrate were compared with the content of the same constituents in the milk produced by a comparable group of cows receiving a normal concentrate mixture. Since any major change in the nitrogen fractions of milk may be expected to influence its manufacturing characteristics, an additional study of the properties of the milk from the foregoing groups included determinations of the nitrogen distribution of the milk.

EXPERIMENTAL

The total nitrogen, the casein nitrogen, the non-casein protein nitrogen and the non-protein nitrogen (7) in the milk produced by the two groups of cows are presented in figure 1.

Immediately after placing the cows on their respective concentrate rations, the non-protein nitrogen in the milk from the cottonseed meal group increased; whereas that from the control group decreased slightly. For the most part, this difference was uniform throughout the remainder of the experimental period. This parallelism was maintained even though there were variations from time to time in the level of the non-protein nitrogen. When the animals were released on pasture, this nitrogen fraction increased, and when silage feeding was resumed, it decreased.

The non-casein protein nitrogen fraction of the milk from the two groups showed no difference that could be attributed to the rations.

During the first four months, no differences were evident in the casein nitrogen content of the two milks. After this period, when a difference in fat and total solids was observed (4), the milk from the cottonseed meal group had a lower casein nitrogen content than the milk from the control

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group. This difference was accentuated when the cows were turned on the pasture, particularly during the first grazing period. The precipitous drop in this constituent noted during this period was associated with a change in the physiological condition of several cows in the cottonseed meal group (4).

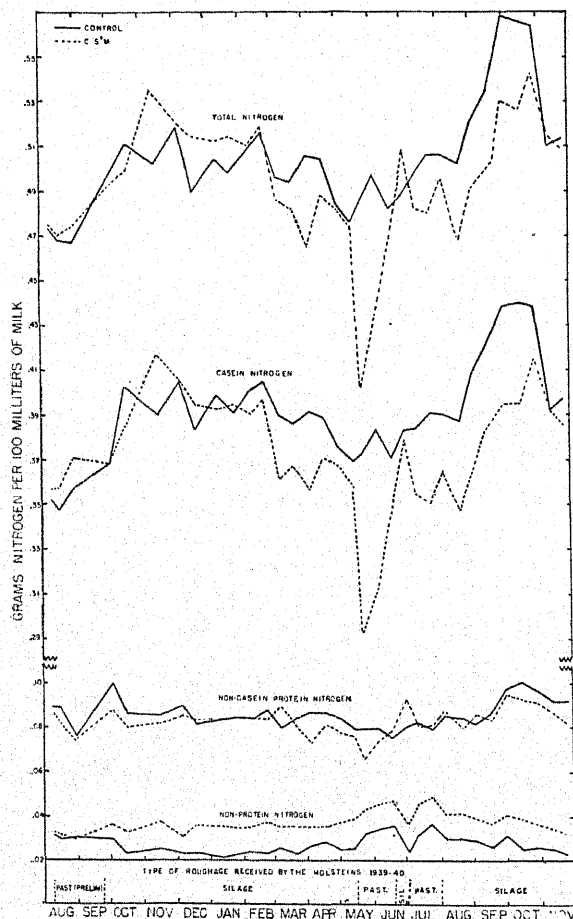


FIG. 1. The amount of total nitrogen, casein nitrogen, non-casein protein nitrogen and non-protein nitrogen in milk produced by a group of cows receiving cottonseed meal as the only concentrate compared with the same nitrogen fractions in the milk produced by a comparable group of cows receiving a normal concentrate mixture.

Apparently the differences in the level of the total nitrogen in the milks from the two groups were due primarily to the relative levels of the non-protein nitrogen and the casein nitrogen fractions. The increase in non-protein nitrogen in the milk from the cottonseed meal group tended to increase the total nitrogen to a higher level than that in the milk from the

control group until changes in the casein nitrogen began to counterbalance the effects of the non-protein nitrogen. The reduction of the casein nitrogen in the milk from the cottonseed meal group apparently was sufficient to offset the higher non-protein nitrogen. Hence, the total nitrogen was first

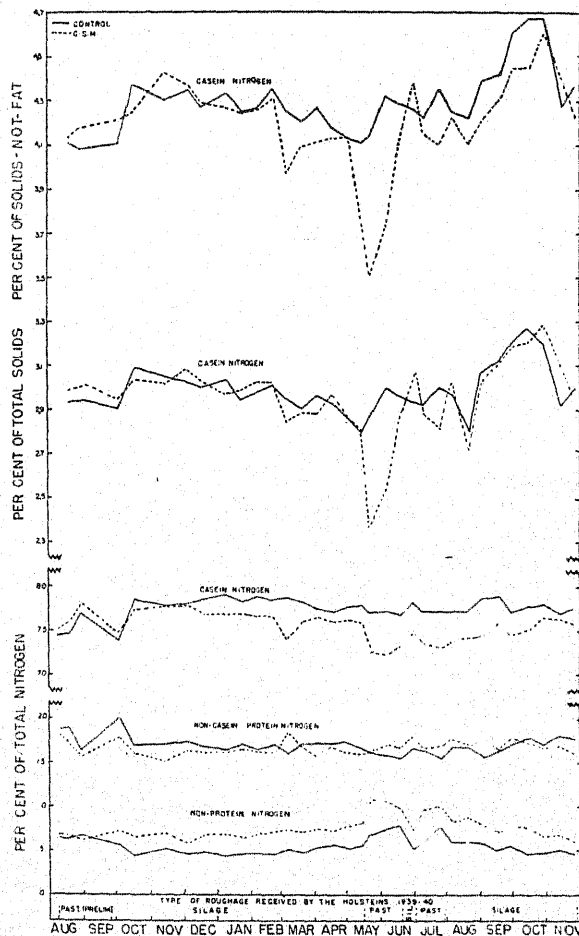


FIG. 2. The per cent of total nitrogen as casein, non-casein protein and non-protein nitrogen, and the per cent of total solids and of solids-not-fat as casein nitrogen in the milk produced by a group of cows receiving cottonseed meal as the only concentrate compared with the same nitrogen distribution in the milk produced by a comparable group of cows receiving a normal concentrate mixture.

higher and then lower in the milk from the cottonseed meal group than in the milk from the control group.

The two sets of curves for casein nitrogen, figure 2, show that this fraction calculated as per cent of the solids-not-fat follows the same trend

and shows the same general group relationships as it does when expressed in terms of grams per 100 milliliters of milk, figure 1. However, when expressed on a basis of per cent of the total solids in the milk, the aforementioned apparent differences between the two milks was not in evidence, except for the period when the cows were physically upset. This indicates that the fat (4) in the milk decreased sufficiently to keep the ratio of casein nitrogen to total solids in the milk from the cottonseed meal group about equal to the same ratio for the milk from the control group.

The remaining three sets of curves presented in figure 2 show the distribution of the total nitrogen as casein, non-casein protein and non-protein nitrogen. The lower percentage of casein nitrogen from the cottonseed meal group, as compared with that of the control group, is due partly to a diluting effect of the increased non-protein nitrogen and partly to a definite lower casein in relation to the solids-not-fat.

DISCUSSION

The high non-protein nitrogen observed in the milk from the group receiving cottonseed meal is in agreement with reports (2, 5, 9, 11) that the level of protein feeding affects directly the amount of non-protein nitrogen present in the milk.

Most of the published reports indicate that the protein nitrogen in the milk is not affected by the level of protein feeding, although a slight increase has been observed. Stewart and Tocher (10) reported that albumin and globulin were increased but that casein was not affected. Hills *et al.* (3) found a slight increase in the casein-albumin fraction with an increasing level of protein feeding. The results reported in this article indicate that the albumin and the globulin apparently were not affected by feed; whereas casein instead of being increased actually was lowered by cottonseed meal feeding.

Riddet *et al.* (6) observed that casein was decreased by subnormal feeding, but the cows in the experiment reported in the present article hardly could be considered on a subnormal ration in terms of total digestible nutrients. Davies (1) found that milk low in solids-not-fat was also low in casein and especially low in lactose but high in non-protein nitrogen, albumin, and globulin nitrogen. If the amount of lactose is calculated from the results reported here (4), the doubtful condition of both a low lactose content and a low ash is shown in the milk from the cows receiving cottonseed meal as compared with milk from the control group.

Using Rowland's (8) value of 77.0 as the lower limit for casein nitrogen, expressed as per cent of total nitrogen for normal milk, the cows on the cottonseed meal ration could be considered as secreting abnormal milk after being on this ration about three months. Rowland has indicated that milk of this type is characteristic of that from cows suffering from sub-

clinical mastitis. Although no special study was made regarding this point, there were no apparent indications that the cottonseed meal group of cows was any more susceptible to udder troubles than the control group.

Results from the groups of Jerseys mentioned in a previous article (4) tended to confirm the effects of cottonseed meal feeding on the non-protein and casein nitrogen of milk as reported in this article.

SUMMARY

The nitrogen distribution of the milk produced by a group of cows receiving cottonseed meal as the only concentrate was compared with that of milk from a comparable group receiving a concentrate mixture of corn gluten meal, wheat bran, and ground corn and oats. The roughage was the same for the two groups.

The milk from the cottonseed meal group had a higher non-protein nitrogen content than that from the control group.

The two systems of feeding resulted in no apparent differences in the non-casein protein nitrogen content of the milk.

About four months after placing the cows on their respective experimental rations, the group receiving cottonseed meal as the only concentrate began to produce milk of a lower casein nitrogen content than did the control group.

The comparatively lower casein nitrogen was accompanied by a lower fat and total solids content in such a ratio that the casein nitrogen expressed as per cent of total solids was the same for both groups.

Differences in the total nitrogen content of the two milks were dependent on the relative levels of non-protein and casein nitrogen; hence the total nitrogen was first higher and then lower in the milk from the cottonseed meal group than in the control group.

The quantity of milk produced by the two groups of cows was essentially the same; therefore the differences in properties of the milk reported in this series of articles cannot be related to the milk yields.

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THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY
CONCENTRATE ON SEVERAL PROPERTIES OF MILK. III.
pH, RENNET COAGULATION, HEAT COAGULATION
AND CURD TENSION*

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Any factor altering the composition of milk may be expected to affect the physical-chemical properties of the milk, while the physical-chemical properties of milk can be altered without any detectable change in milk composition. For instance, an affect on the rennet coagulation of milk may or may not be accompanied by a detectable change in milk composition (5, 6, 11, 13).

The belief that feed does not directly affect the composition of the milk has led to the general opinion that such properties as pH, rennet coagulation, heat coagulation, and curd tension are likewise not affected by the ration. Investigations have shown that acids in the ration do not alter the acidity of the milk (1, 10). Neither factors affecting the curd tension of milk (2) nor other coagulation characteristics of milk have been definitely related to the feed consumed by the cow.

In preceding reports the total solids, the fat and the ash content (8) and the nitrogen distribution (9) in the milk produced by a group of cows receiving cottonseed meal as the only concentrate were compared with the same properties in milk produced by a comparable group of cows receiving a normal concentrate mixture. The cottonseed meal group of cows produced milk of a lower solids content than the control group; hence, other properties of the milk might be expected to have been affected. Thus additional data from milk samples previously described (8, 9) are presented in this report.

EXPERIMENTAL

The hydrogen ion concentration of the milk samples was determined with a glass electrode pH meter. The rennet coagulation time was obtained by the procedure as outlined by Sommer and Matsen (12). The heat coagulation was studied by recording the time required for the milk to coagulate when sealed in small glass tubes and immersed and agitated in an oil bath at 136° C. (7). The effect of small changes in the salt balance of the milk on the heat coagulation was determined by recording the time required for coagulation of various milk samples to which calcium acetate and sodium diphosphate, respectively, had been added at the rate of 0.4 ml. of M/4 salt per 50 ml. of milk. The procedure recommended by the Committee on

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Methods of Determining the Curd Tension of Milk (3) was used for studying the curd tension of the milk samples. In addition to the determination of the maximum grams required for cutting the surface the weight for cutting the body of the curd also was recorded.

A comparison of the pH, the rennet coagulation, the heat coagulation and the curd tension of the respective milks produced by the two groups of cows is presented in figure 1.

The milk from the cottonseed meal group had a slightly but consistently higher pH than the milk from the control group during the first few months

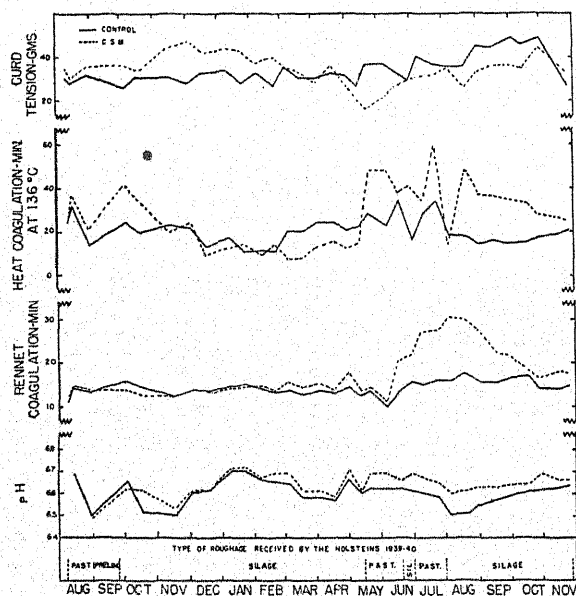


FIG. 1. The pH, rennet coagulation, heat coagulation and curd tension of the milk produced by a group of cows receiving cottonseed meal as the only concentrate as compared with the same properties of the milk produced by a comparable group receiving a normal concentrate mixture.

of the feeding trial. The change from silage feeding to pasture grazing was accompanied by a still greater spread in the pH values of the two milks, the increased difference persisting throughout the remainder of the trial.

After the first four months, when the milk from the cottonseed meal group began to have a lower solids content (8), the rennet coagulation time for this milk began to be slightly greater than for the control milk. A few weeks after turning the cows on pasture the rennet coagulation time for the milk from the cottonseed meal group increased markedly over a period of about three months and then gradually returned to near the normal level after silage feeding was resumed.

The heat coagulation of the milks showed no uniform and definite differences; however, when the cows were changed back and forth from pasture to silage, the heat coagulation of the milk from both groups fluctuated considerably, the milk from the group on the cottonseed meal ration tending to be more stable to heat than that from the control group.

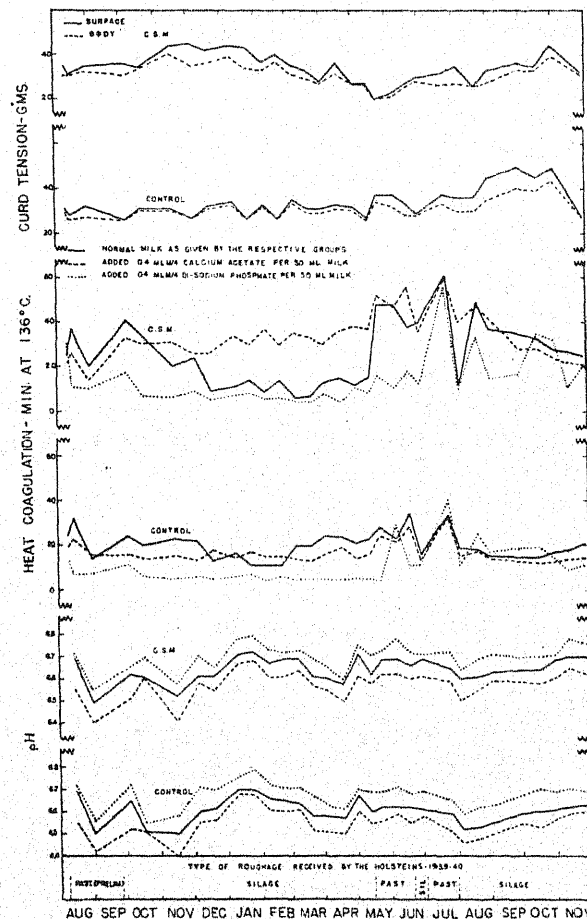


FIG. 2. The effects of added salts on the pH and the heat coagulation, and the differences between surface and body curd tension of the milks produced by the groups of cows on a cottonseed meal and control rations.

During the first few months the curd tension of the milk from the cottonseed meal group was higher, but for the remainder of the trial it was lower than that from the control group. There seemed to be a direct relationship between the lower solids content of the milk (8) and the lower curd tension. As shown in figure 2 there is a marked parallelism between the surface curd tension and the body curd tension in the respective samples of milk.

The additions of calcium acetate and of di-sodium phosphate to the milks had the same effect on the pH of the two milks, but different effects on the heat coagulation as is indicated by the curves presented in figure 2. The addition of phosphate always increased and the addition of calcium always decreased the pH of the milk samples. The heat coagulation time for the milk from the control group usually was decreased by the addition of either salt; whereas the addition of the calcium salt usually increased while phosphate decreased the heat stability of the milk from the cottonseed meal group.

DISCUSSION

The general differences between the milk from the cottonseed meal group and that from the control group in pH, rennet coagulation, heat coagulation and curd tension follow, for the most part, trends that could be predicted from the differences in the composition of the milks (8).

The higher pH of the milk from the cottonseed meal group in comparison with the milk from the control group is in accord with the lower solids-not-fat content of the cottonseed meal milk. Keith *et al.* (4) observed these same general differences in a comparison of milk produced by a group of Jerseys receiving cottonseed meal and prairie hay with that from a similar group receiving a standard herd ration.

The lower solids in the milk from the cottonseed meal group would account for a longer rennet coagulation time than for the milk from the control group. The difference in ash content of the two milks is also in harmony with the differences in rennet coagulation time. The marked increase in coagulation time for the group from the cottonseed meal group occurred following a physical upset of the cows on the cottonseed meal ration (8). This prolonged increase in the rennet coagulation time was probably associated with the physiological condition of the cows.

The effects on the heat coagulation of the milks from the two groups were variable, but the results revealed a difference in the heat stability. The differences in coagulation time and in composition of the comparative whole milks suggested that a difference in the heat stability of their evaporated products might be expected.

SUMMARY

The pH, rennet coagulation, heat coagulation and curd tension of the milk produced by a group of cows receiving cottonseed meal as the only concentrate were compared with the same properties of milk produced by a comparable group of cows receiving a normal concentrate mixture.

These properties of the milks follow the same general trend as the differences in composition of the two milks.

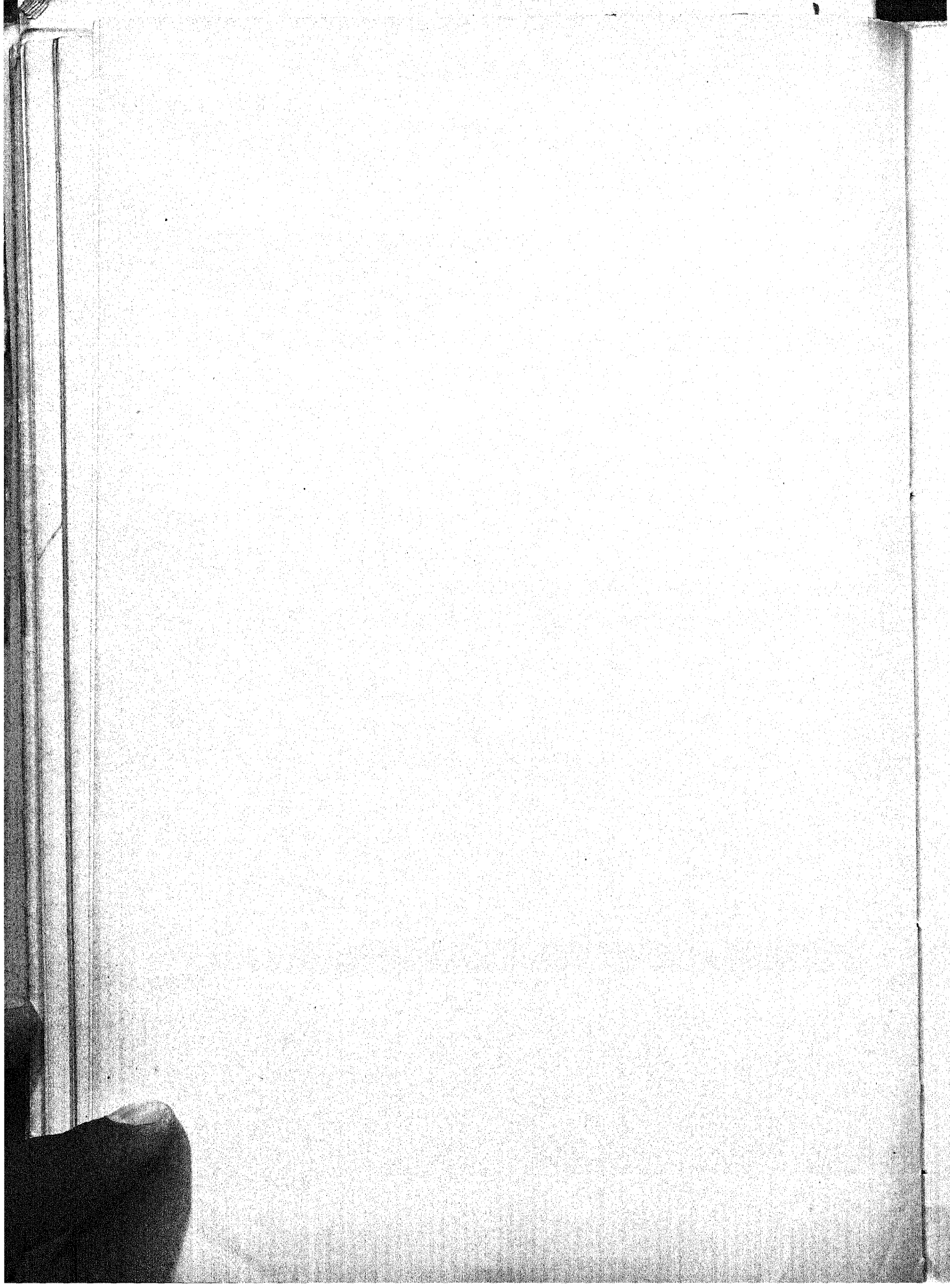
The milk from the cottonseed meal group had a higher pH than that from the control group. The slight difference observed during the first few months increased as the difference in total solids increased.

The milk from the cottonseed meal group required longer to coagulate by rennet than that from the control group after about the first five months of the feeding trial.

Variable differences were recorded for the heat coagulation of the milks. The data suggest a practical problem relative to the heat stability of the evaporated products produced from milk of cows receiving a high level of cottonseed meal in the ration.

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THE EFFECT OF FEEDING COTTONSEED MEAL AS THE ONLY CONCENTRATE ON SEVERAL PROPERTIES OF MILK.

IV. FAT CONSTANTS*

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Numerous reports in the literature show that many feeds affect the composition of milk fat. A great number of these records are concerned with cottonseed and cottonseed products as dairy feeds. Although most of the investigations recorded are in agreement relative to the specific effects of cottonseed meal upon the fat properties, the few discrepancies noted may be explained partially on the basis of the role played by such factors as length of feeding period, type of basal ration, method of comparison, etc., on the nature and degree of alteration of milk fat.

Most of the studies of the effect of feeding cottonseed meal on milk fat composition have been for short periods, and many of the investigators have given little consideration to the type of basal ration and to the maintenance of adequate control groups of cows.

Since the composition of the fat is known to be influenced easily by feeds and since the effects of cottonseed meal feeding over a short duration of time have been fairly well established, it was deemed desirable to determine the comparative effects of protracted cottonseed meal feeding on some of the constants of the fat from the milk samples obtained under conditions previously reported (2, 3, 4). Furthermore, since the composition of the fat has been shown to be related to its susceptibility to oxidation (5), observations were made on the comparative effects of the feeds on the oxidation of filtered milk fat.

EXPERIMENTAL

The fat samples were obtained from milk produced by two comparative groups of cows, one of which received cottonseed meal as the only concentrate, while the other received a normal grain mixture. The respective milk samples were pasteurized and churned. The resulting butter was then melted and filtered for subsequent study.

The saponification number, iodine number, Reichert-Meissl number, butyro-refractometer degrees, stability value (5) and acid degree of the fat samples were determined. The data obtained are presented in figure 1 and in table 1.

The milk fat from the cottonseed meal group had a lower saponification number than that from the control group, a relationship which would be

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predicted from a review of the literature. However, for a short period during the sixth and seventh months of the feeding trial this difference was practically nil. It is not known whether the latter deviation from the expected results was due to the ration or to some other cause.

For most of the feeding period the milk fat from the cottonseed meal group had a higher iodine number than that of the control group. During the seventh month, the iodine number, as did the saponification number, failed to show the expected difference in the two milk fats. While the cows were being transferred back and forth from silage to pasture, the differences

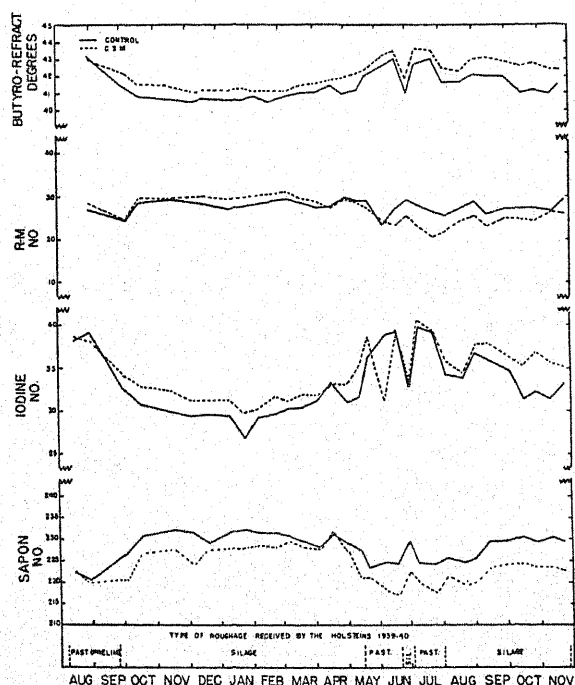


FIG. 1. The saponification number, iodine number, Reichert-Meissl number and butyro-refractometer degrees of the milk fat produced by a group of cows receiving cottonseed meal as the only concentrate as compared with the same fat constants of the milk fat produced by a comparable group of cows receiving a normal concentrate mixture.

in the iodine number were variable, which is in harmony with the results presented in the literature. The iodine number is easily influenced not only by the concentrate but also by the type of roughage. The effects of a change in roughage on the iodine number depend upon the concentrate fed; hence, the variable results obtained during these sudden changes in roughage might have been expected.

The Reichert-Meissl numbers obtained show the greatest deviation from the expected. During the first nine months of the feeding trial, these values

for the milk fat produced by the two groups were practically the same. For the remainder of the trial the milk fat from the cottonseed meal group had the lower Reichert-Meissl number as expected. Although the effects of oil feeds are known to be modified by silage and by pasture, neither this nor any other known factor explains adequately the long time required for the cottonseed meal to affect the volatile acids of the milk fat.

The refractive index was the only fat constants that revealed a consistent difference between the milk fats from the two groups of cows. The milk fat from the cottonseed meal group always had a higher refractometer reading than that of the control group. Most of the reports in the literature show a similar increase of the refractive index resulting from the feeding of cottonseed and its products.

TABLE 1

The stability value and acid degree of milk fat produced by cows receiving cottonseed meal as the only concentrate compared with milk fat from cows receiving a normal grain mixture

Date sampled	Stability value		Acid degree	
	C.S.M.	Control	C.S.M.	Control
7-24-40	27.5	32.0	0.42	0.56
8- 6-40	34.5	33.5	0.65	1.35
8-20-40	32.0	33.0	0.57	0.60
9- 3-40	32.0	36.0	0.54	0.78
9-17-40	28.5	39.0	0.62	0.72
10- 1-40	26.0	32.0	0.70	0.57
10-15-40	21.0	25.0	0.60	0.58
10-29-40	28.0	35.0	0.58	0.58
11-12-40	18.0	30.0	0.74	0.54
11-26-40	34.0	37.0	0.56	0.43

A number of factors are known to affect the values obtained in the determination of the susceptibility of fat to oxidation. After eliminating the possibility of the usually recognized sources of error, it was found that an additional factor, the presence of free fatty acids, had a greater influence on fat oxidation than anticipated (1). Before the effect of feed could be measured, it was necessary to eliminate the possibility of even slight lipolytic action on the milk fat.

A few comparisons of the stability value (5) and of the acid degree of the milk fat produced by the two groups of cows are presented in table 1. The milk fat from the cottonseed meal group was found to be less stable toward oxidation than that of the control group. This agrees with investigations showing that the stability of filtered fat varies with the degree of unsaturation. No consistent difference was observed in acid degree between the two milk fats. Unpublished preliminary work on the determination of free fatty acids in milk shows that the churning method probably is not a reliable procedure for obtaining fat for use in measuring the free fatty

acids in milk. Thus the acid degrees presented herein may or may not represent the true relative values.

SUMMARY

In general the feeding of cottonseed meal as the only concentrate as compared with feeding a normal grain mixture decreased the saponification number and increased the iodine number and the refractive index. The expected decrease in Reichert-Meissl number did not become evident until after nine months of the feeding period had passed.

In a similar comparison, the milk fat from the cottonseed meal group was less stable toward oxidation than that from the control group. No difference in acid degree of the two milk fats was detected.

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THE CORRELATION BETWEEN SOME CHARACTERISTICS OF DAIRY BULL SEMEN AND CONCEPTION RATE*

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The accurate evaluation of freshly drawn and stored bull semen before use is of prime importance in artificial insemination. The detection before use and discarding of semen samples likely to result in poor conception rates is most important in securing maximum efficiency. The physical and chemical characteristics which have been commonly proposed for the evaluation of semen are pH, concentration, motility, viability in storage, percentage of abnormal sperm, glycolysis rate, respiration rate, reduction tests, resistance to various shock treatments, percentage of live sperm, etc. This paper reports a study of the correlation between the first five of the characteristics listed above and the conception rate of the semen as used in the University of Missouri Dairy Herd.

MATERIALS AND METHODS

The semen studied was largely from University of Missouri bulls of the Holstein-Friesian, Guernsey and Jersey breeds. Cows inseminated were nearly all in the University of Missouri herd, but a few cows and bulls in outside herds have been included in the data. Semen from twenty-three bulls was studied. The bulls studied represented all phases of fertility from practically sterile to highly fertile. The bulls ranged from fifteen months to thirteen years of age.

All inseminations were made by cervical deposition by the use of a speculum as described by Herman and Ragsdale (4). Pregnancy determinations have been supported in most cases by calvings, and in others by manual examinations for presence of fetus or failure of cow to return to heat within 90 days after insemination. The data cover the years 1940 to 1942 and the early part of 1943. Insemination of cows which never conceived or were known to have malfunction of the ovaries or other reproductive trouble were omitted from the study.

The methods used in examination of the semen have been described previously (5). These were simple tests which could be done satisfactorily with little technical skill. The rating of motility from 0 to 5 lacks somewhat in precision, and further study of accurately counting the motile sperm shows that a small number of very active spermatozoa may make the semen appear as good in motility as a larger number of slowly moving sperm.

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Since the vigor of motion as well as the amount is an important test of fertility (2, 7), the rating method may be of great value in spite of its error in estimating the actual number of sperm which are motile. Actual counts have shown that the previously estimated percentage of motile sperm for the different ratings was too high. Semen of 5 motility has about 80 per cent or more progressively motile sperm; 4 motility, 70 to 85 per cent; 3 motility, 45 to 75 per cent; 2 motility, 20 to 50 per cent; and 1 motility is usually below 25 per cent of progressively motile sperm.

Since two or more cows were seldom inseminated with any one sample of semen, the data were grouped to facilitate correlation analysis. All inseminations were tabulated in class interval groups of the semen characteristic as to whether or not they resulted in conception, and the percentage of samples resulting in conception was calculated for each group. The coefficients of linear correlation, r , were then determined for the various semen characteristics and conception rate.

RESULTS

The results secured are presented graphically in figure 1 for all of the characteristics studied except pH. The importance of pH readings was in doubt early in the period covered, hence the pH of all samples was not determined as a routine procedure. The pH of semen used for 205 inseminations was recorded, however. These were grouped in classes of 0.2 pH unit interval from pH 5.8 to 7.4. The correlation coefficient, r , for pH and conception rate was -0.18 which indicated that there certainly was not a correlation between the two.

The percentage of abnormally shaped sperm in semen used in 525 inseminations was tabulated in groups at intervals of 3 per cent from 0 to 30 per cent. As shown by the chart (fig. 1) and the correlation coefficient of -0.12 there was no correlation between conception rate and percentage of abnormal spermatozoa in the range studied. Bulls producing extremely large numbers of abnormally shaped sperm, however, may be low in fertility (5).

The concentration of sperm in semen used in 559 inseminations was tabulated in groups at intervals of 200 per mm.³ from 200 to 2000. As shown in figure 1 there was a slight tendency for the more concentrated semen to produce a larger percentage of conceptions. The correlation coefficient, 0.63, was just short of significance, since an r of 0.666 is required (3) for the expression of a significant correlation within a probability of 0.05. The concentration of sperm was, therefore, not an important factor in the determination of conception rate.

Semen used in 475 inseminations was tabulated as to the length of time a motility rating as good as 2 was maintained in the undiluted semen during storage at 40° F. Daily motility ratings of the stored semen were made; so the samples were grouped at intervals of 24 hours up to 192 hours. Forty-one per cent of inseminations from semen that kept a motility rating of 2 for

less than one day after use produced conceptions. As viability of the semen increased the conception rate increased until 68 per cent of inseminations with the most viable semen group resulted in conception. The correlation coefficient, 0.84, showed that there was a highly significant linear correlation between viability of the sperm in storage and their ability to produce pregnancy.

The motility ratings of semen used in 565 inseminations were tabulated according to the fertility of the semen. As shown in figure 1, plotting the motility ratings against conception rate did not indicate a linear correlation.

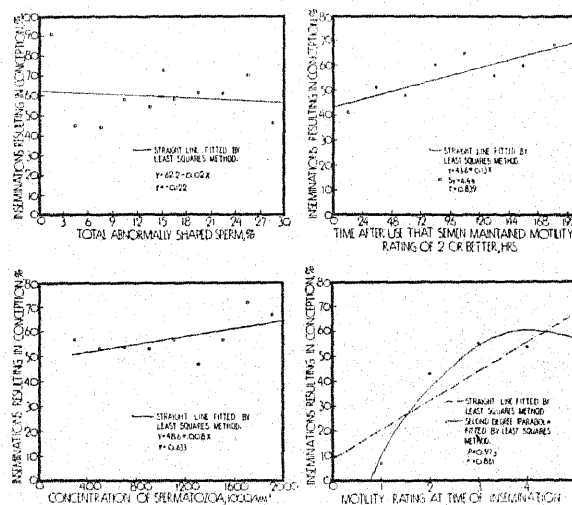


FIG. 1. The correlation between conception rate and semen quality as expressed by motility estimation, viability, concentration of sperm, and abnormal morphology of sperm.

Conception rate from semen of 1 motility was very poor. Semen that was 2 motility was not very satisfactory since only 43 per cent of such inseminations resulted in conception. Semen of 3 motility was correspondingly better than semen of 2 motility as 2 had been better than 1. Conception rate of semen which was rated 4 and 5, however, was not significantly different from that of semen rated only 3, although semen rated 5 motility did give the best conception rate. The relation between motility rating and conception rate was therefore curvilinear. Conception rate increased as motility increased up to the 3 rating, and thereafter increased motility did not result in important increases in conception rate. The index of correlation, ρ , should therefore be used to measure the degree of correlation between the two. The index of correlation, 0.97, revealed that there was a significant curvilinear correlation between motility rating and conception rate.

DISCUSSION

This evaluation of five commonly used methods of determining semen quality has revealed some useful information for the practitioner. Accord-

ing to the results of this study there is little practical value in making routine pH determinations, abnormality counts, or determining the concentration of sperm. The information furnished by these determinations was not correlated significantly with rate of conception. Therefore, the rejection of a sample of semen because it is slightly abnormal in any of the three characteristics does not seem justified. The only reason for rejecting samples which are very high in pH or abnormalities or very low in concentration should be the extent to which such items are correlated with longevity and motility of the sperm. This problem has not been treated in this study. If samples are of acceptable motility and viability, it does not seem reasonable to reject them on the basis of pH and concentration, especially in view of the fact that the semen may be diluted many times with a buffered protective mixture. Semen that is very high in abnormal sperm usually does not have good motility or viability; but if it does have good motility and viability it will probably give good breeding efficiency.

The fine correlations shown between viability and conception rate are of more theoretical interest than practical interest because such information is not obtained until after use of the semen. This correlation can be used as a guide, however, in the development of other methods of semen evaluation. The high correlation of any test with viability should indicate its correlation with semen fertility. Beck and Salisbury (1) have used short-time high-temperature survival tests in this manner. Knowledge of the probable survival time of highly motile semen is important for selection of semen which must be stored as well as for the maintenance of good conception rates.

The curvilinear relationship between conception rate and motility rating of the semen at the time of insemination will be of special interest in the use of stored semen. Semen which is below a rating of 3 cannot be used with much confidence. Even though many pregnancies have resulted from semen of lower motility the chances of conception are so reduced that such practice may be worthwhile only in the case of very valuable sires. The data also indicate that practically as good results may be expected from semen of 3 and 4 motility as from the highest rating. These observations are in agreement with those of Lasley (6) who found no correlation between motility rating above 3 and the fertility of the semen. He also observed that there was not a significant difference in the fertility of semen containing from 55 to 95 per cent live sperm, but semen which contained only 20 per cent of live sperm was infertile. It therefore seems safe to conclude that semen should be 3 motility or better if good breeding efficiency is to be maintained. In terms of progressively motile spermatozoa, there should be about 45 per cent or more. This study has again demonstrated the value of the motility rating for detecting semen likely to result in poor breeding efficiency. Further study of the value of accurately determining the percentage of progressively motile sperm with reference to its correlation with viability is in progress.

SUMMARY

A study has been made of the correlation between semen characteristics and conception rate in the University of Missouri dairy herd. The correlation between conception rate and pH, abnormal sperm, and concentration of sperm was found to be non-significant. A highly significant linear correlation was found between conception rate and viability of the sperm. A significant curvilinear relationship between motility and conception rate was found. The difference in conception rate between semen rated 3 motility, which is usually 45 per cent or more progressively motile sperm, and higher grades of motility was very slight.

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SEASONAL VARIATION IN SEMEN QUALITY OF SOME MISSOURI DAIRY BULLS*

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The seasonal nature of sexual activity of many mammals is well recognized, but whether or not the bull has a definite seasonal sexual cycle has not been clearly demonstrated. Morgan and Davis (8) found that the conception rate of Nebraska dairy cattle was lowest in August and September, but months with high conception rate were not seasonal since January and July were both high and December was highest in conception rate. Erb, Wilbur and Hilton (5) reported a seasonal variation in breeding efficiency with spring (May at maximum) giving the best conception rate and late summer (August at minimum) giving the lowest conception rate. The separate effect of the bull and the cow upon fertility are confounded in ordinary analysis of breeding records, however.

Few studies have been made of seasonal variations in semen quality. Weatherby, Reece and Bartlett (11) reported monthly averages of semen volume, concentration, and longevity for six bulls in a New Jersey cooperative. Longevity, which is correlated with fertility (10), was generally higher in June and July than in other months, but the differences among the months were very slight. Anderson (1) presented monthly average semen volume and motility observations made on bulls in Kenya which indicated a rather distinct seasonal tendency. Volume and motility were both low from May to August. Erb, Andrews and Hilton (4) made a systematic study of the effect of season upon semen characteristics. Except for a noticeable increase in concentration in the spring, seasons other than summer had little effect upon semen quality. During July, August, and September the motility and semen volume were lowered, the sperm survived for a shorter period, and the percentage of abnormal forms was increased. Statistical analysis showed that semen obtained during the summer months was significantly of lower quality than that of other seasons. Lasley (7) found that beef bulls in Arizona gave higher quality semen as the season progressed from May to September. Volume of semen, percentage of live sperm, and percentage of resistant sperm increased during this period but concentration and percentage of abnormal sperm did not change.

The literature in general is not in complete agreement concerning the effects of season upon semen quality of bulls. The explanation of this fact may be that the effect of season is not as great in some localities as in others.

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In many cases the bulls studied were few in number and their individual responses to season (4) may not have been typical of the population. Since January, 1940, semen of a number of bulls in the University of Missouri herd has been examined regularly. Data for ten of these bulls extended over all four seasons, some of them for two or more years, and from three others, data from two or more seasons were available. This report presents an analysis of the monthly variation in quality of semen produced by these thirteen dairy bulls.

MATERIALS AND METHODS

The bulls were all purebred animals used as herd sires in the University of Missouri herd. Nine were Holstein-Friesians, three were Jerseys, and one was a Guernsey. They varied in age from one and one-half to thirteen years, but the majority of them were aged bulls, five year old or older. The average age was six years. Although these bulls were not all of high fertility, none was of consistently very low fertility.

During 1940, semen was collected from each bull at least once each week. Thereafter, however, collections were made only as they were needed for breeding or for various investigational purposes. All bulls were not represented, therefore, in every month of every year included in the study.

Each sample of semen was examined for initial motility, concentration, volume, number of abnormal sperm, and viability according to methods described previously (6). The pH of many of the semen samples was also determined (6).

The observations made on the semen from each bull were averaged by months, and the monthly averages for the separate bulls were then averaged to secure the grand average for that month. If data were available for a certain bull for two or more years for any month, each year's data were handled as a separate monthly average. Statistical significance of the differences among months and among bulls was determined by the analysis of variance according to Snedecor (9).

The bulls were fed a good quality legume hay and a grain mixture the year around. No pasture or green feed was given and no silage was fed. There was no seasonal difference in the rations.

RESULTS

Observations on 1103 ejaculates were included in this study. These were separated into 254 bull-month averages, resulting in an average of 4.34 ejaculates per month per bull. The largest number of ejaculates taken from one bull in any month was 12. Many bulls were collected from only once in certain months.

The monthly averages are presented in table 1. The number of bull-month averages from which the monthly average was computed and the

number of bulls represented are listed below the average of each month. The monthly averages are also presented graphically in figure 1.

The seasonal averages are presented in table 1. Winter was taken as January, February, and March. Spring months were April, May, and June. July, August and September were taken as summer; and October, November and December were designated fall months.

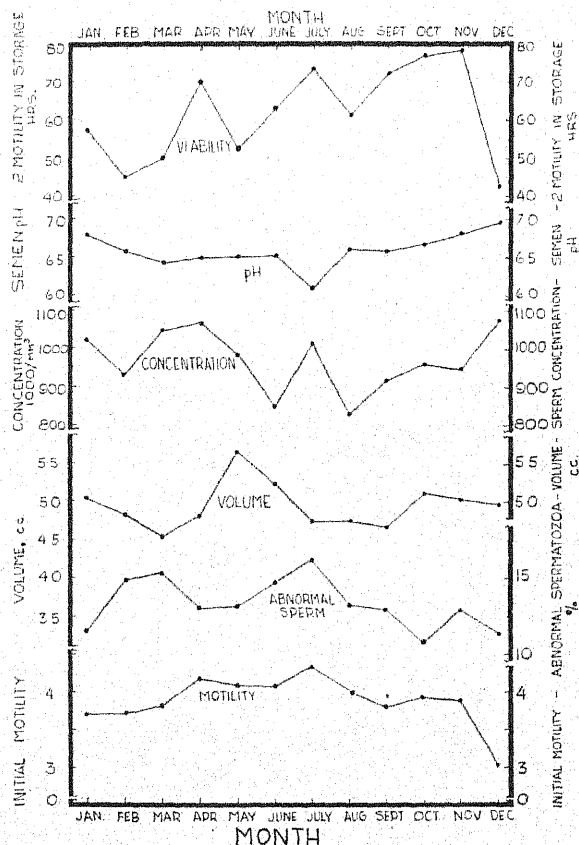


FIG. 1. Monthly averages of examinations for quality of semen from 13 Missouri bulls during 1940, 1941, and 1942.

The data in table 1 and the curves in figure 1 do not indicate very definite seasonal variations of some semen characteristics, but there is a suggestion that others may vary with the seasons. Volume appears to be greater in spring months, initial motility and viability are low in winter months, and pH is lower in summer than in winter. An examination of the original data revealed marked variation among bulls for certain characters in addition to variations among months by the same bull. Furthermore, all bulls did not

exhibit the same sort of seasonal variation. The nature of the data did not permit an accurate assignment of all of the sources of variation by the

TABLE 1
Monthly and seasonal summary of semen qualities

		Volume	Concentration	pH	Initial motility	Maintenance of 2 motility	Abnormal spermatozoa
		cc.	1000/mm. ³			hrs.	%
January	Mean	5.04	1023.0	6.79	3.71	57.4	11.43
	No.	27	27	17	27	26	26
	Bulls	13	13	11	13	13	12
February	Mean	4.83	931.5	6.57	3.71	44.7	14.82
	No.	24	24	13	24	21	20
	Bulls	11	11	8	11	11	11
March	Mean	4.53	1046.1	6.42	3.81	49.3	15.29
	No.	24	24	10	24	23	20
	Bulls	12	12	8	12	12	12
April	Mean	4.80	1066.7	6.49	4.18	69.8	12.96
	No.	21	21	14	21	21	21
	Bulls	12	12	10	12	12	12
May	Mean	5.65	984.6	6.50	4.09	52.1	13.08
	No.	21	21	12	21	19	21
	Bulls	11	11	8	11	11	11
June	Mean	5.21	851.3	6.52	4.07	62.9	14.65
	No.	20	20	15	20	18	20
	Bulls	12	12	10	12	12	12
July	Mean	4.73	1015.9	6.09	4.33	73.8	16.21
	No.	18	19	7	19	19	18
	Bulls	11	11	7	11	11	11
August	Mean	4.75	831.3	6.60	4.02	61.5	13.25
	No.	17	15	7	17	14	15
	Bulls	10	10	7	10	10	10
September	Mean	4.67	917.9	6.58	3.81	72.1	12.93
	No.	21	21	7	21	21	21
	Bulls	11	11	7	11	11	11
October	Mean	5.12	961.0	6.68	3.94	77.2	10.75
	No.	21	21	11	21	21	21
	Bulls	11	11	9	11	11	11
November	Mean	5.04	944.7	6.81	3.90	78.8	12.96
	No.	18	19	13	19	18	18
	Bulls	11	11	9	11	10	11
December	Mean	4.98	1075.1	6.96	3.07	42.7	11.47
	No.	19	20	7	20	19	20
	Bulls	9	10	6	10	9	10
Year	Mean	4.95	975.7	6.60	3.88	61.6	13.23
	No.	251	252	133	254	240	241
	Bulls	13	13	13	13	13	13
Winter	Mean	4.80	1000.2	6.59	3.74	50.5	13.85
Spring	Mean	5.22	967.5	6.50	4.11	61.6	13.56
Summer	Mean	4.72	921.7	6.42	4.05	69.1	14.13
Fall	Mean	5.05	993.6	6.82	3.64	66.2	11.73

analysis of variance method (9). Variation among months and variation among bulls, however, were calculated and their significance was determined.

Volume. The differences in volume of ejaculates among bulls were highly significant. This finding was to be expected because bulls differ widely in this respect. The inter-monthly volume variations were not significant, however; hence a significant seasonal variation of semen volume was not found. Differences as great as were observed could have occurred at least 1 in 20 times as a result of chance. Thus, although the data are suggestive of a greater volume of ejaculate produced in spring than summer, the average difference of 0.5 cc. could easily have occurred as a result of chance.

Concentration. The differences in sperm concentration among bulls were highly significant. The differences among months, however, were not significant since they could be expected as a result of chance slightly more than 1 in 20 times. The same was true of the inter-seasonal differences. The concentration of sperm was, therefore, not significantly lower in summer than in winter.

pH. The pH differences among months were significant statistically. At $p = 0.05$ July's average was significantly lower than all other months, and December's average was significantly higher than all months except January, October, and November. The difference between the summer average and the fall average was highly significant ($p = < 0.01$). The pH of fall and winter semen seemed definitely higher than the pH of summer semen, but most of the difference was due to the very low values secured in July.

Initial motility. The differences in initial motility among the different bulls were highly significant. The differences among months were also highly significant. Using $p = 0.01$ as the level of significance the initial motility of July semen was significantly higher than that of December, January and February; and December was significantly below all other months. Using the lower level, $p = 0.05$, as a level of significance, April initial motility was significantly above that of December, January and February; July was significantly better than November, December, January, February, March and September; and December was significantly below all other months. Grouping the months into seasons (table 1) showed that spring and summer semen had a significantly higher motility than fall and winter semen. The difference between fall and winter semen was not significant, and spring and summer semen did not differ significantly in initial motility. The semen collected during the warm months definitely had more vigorous motility than that collected during the cold months of fall and winter.

Maintenance of useful motility in storage. The storing ability of the undiluted semen showed significant difference among the bulls and among the months. With $p = 0.01$ representing the level of significance, the storage time of December was significantly below July, September, October, and November; July, October, and November were significantly above February;

and, October and November were significantly above March. When $p = 0.05$ was taken as the significant point, December and February storage was significantly below that of April, July, September, October and November; and February, March, May and December were significantly below October and November. When the months were grouped into seasons, the average storage time of the winter months was significantly lower than that of the summer and fall months, but the differences between winter and spring months may have occurred as a result of chance 1 out of 20 times. The storing ability of the semen was markedly depressed by the cold months, December to March, and it improved in general after that time to reach its peak in late summer and early fall.

Abnormal spermatozoa. The differences in abnormal sperm production among bulls were highly significant. The variation among the bulls was so great that the small differences among the months were not significant. The greatest percentage of abnormal sperm was produced in summer and the least in the fall. Grouping the months into seasons made the difference between summer and fall on the borderline of significance ($p = 0.05$). The other seasons did not differ significantly from each other in any combination, hence the importance of the difference observed between morphology of summer and fall semen is considered to be very slight.

DISCUSSION

The most significant seasonal change in the average semen quality of the bulls studied was a decrease in initial motility and viability and an increase in pH with the onset of cold weather in December. These indications of poorer quality semen were probably due to the poorer physical vigor or inadequate sexual stimulation of the old bulls in cold weather. These changes were confined almost entirely to the bulls which were over four years old. The semen from young bulls was as good in motility and viability in December and January as it was in June and July. Since the ejaculates from young bulls were a small minority in this study, however, the averages exhibit more nearly the picture presented by older bulls. Erb *et al.* (4) also have noted the deleterious effect of very cold weather upon semen quality. Since proved bulls which are to be used widely in artificial inseminating work will usually be fully as aged as the bulls used in this study, the results secured should be borne in mind in caring for them. The breeding chute and collection stall at the University of Missouri are out in the open, unprotected from the elements. The bull sheds are three-sided structures, open to the east. The old bulls were noticeably less vigorous and less eager to serve during cold weather than during warm weather and were somewhat "crampy" in winter. It is probable that greater attention to the comfort of the aged sires would result in improved quality of the winter semen.

The drop in semen quality in summer observed by Erb *et al.* (4) and Anderson (1) did not occur. The heat of summer apparently did not ad-

versely affect spermatogenesis in these bulls. In fact, excellent quality semen was obtained regularly from all bulls during the warmest season. These results concur with the observations of Weatherby *et al.* (11) and Lasley (7). The only indication of a poorer quality of sperm produced in summer was the increased abnormal forms produced in July. Since the percentage of abnormal sperm decreased during August and September, also hot months, it does not seem logical to correlate the increase observed in July with temperature.

It was also observed, in confirmation of Erb's (4) observations, that the volume of the ejaculate and the concentration of spermatozoa were slightly lower in summer than in other seasons. The differences were not statistically significant, however; and it is believed that the decreases were not an indication of lowered spermatogenesis or fertility. Motility and viability of the spermatozoa which are closely correlated with fertility, were above average in the summer months.

The low pH for July was due to a few early determinations in that month which were made on semen which had not been cooled promptly. The air temperature was so high that the metabolism of the sperm was kept at a high rate with a resulting high acid production. It is believed that a larger body of data secured under as uniform controlled conditions as those for August and the following months would not show pronounced monthly variations in semen pH. Anderson (2) did not find a significant monthly variation in the pH of bull semen.

Considering altogether the six characteristics of bull semen which have been discussed, it does not seem that spermatogenesis in the dairy bull is significantly affected by season. Furthermore, in the young, vigorous bulls under observation no important seasonal effects were observed. The aged bulls seemed to suffer more from cold weather than the younger bulls and showed less sexual drive or vigor in winter. The general lowered physical vigor possibly was reflected in decreased vitality of their spermatozoa. It seems probable, therefore, that season as such may have no important effect upon the semen quality of dairy bulls in Missouri. Environmental conditions that may adversely affect the physical comfort or even the vitality or health of the bull, however, may result in the production of poorer quality semen. The effect of the observed seasonal differences in semen quality upon fertility of bulls used for natural service or for artificial insemination with non-stored semen would probably be insignificant because of the slight difference in conception rate which has been demonstrated between medium and high quality semen (10). Dawson (3) found no significant seasonal differences in the fertility of aged sires from widely distributed stations in the United States used for natural service. Where artificial insemination practices requiring regular collection and storage of high quality semen are followed, the effect of extreme weather conditions

or other factors adversely affecting the physical vigor of old bulls may be responsible for varying conception rates.

SUMMARY

A study of the monthly variation of the initial motility, volume, concentration, useful storage time, pH, and morphology of the semen of thirteen dairy bulls used in the University of Missouri herd during three years has been presented. The monthly variations in volume, concentration, and percentage of total abnormal spermatozoa were not statistically significant. The pH of the semen was significantly lower in the summer than in the fall. Initial motility and useful viability were lower in winter than in spring and summer. The results were interpreted as being largely due to the adverse effect of winter weather upon the physical well being and sexual activity of the aged bulls which furnished the majority of the semen studied.

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WHEAT GERM OIL AS AN ANTIOXIDANT IN DAIRY PRODUCTS

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The use of antioxidants in the manufacture of food stuffs containing oils and fats has been studied by a number of investigators. Greenbank and Holm (13) found maleic acid, hydroquinone, and certain other phenolic compounds to have antioxidant properties. Chilson (3), Dahle and Palmer (10), and Greenbank (12) reported that the addition of 20 to 100 milligrams of pure vitamin C per liter of milk retarded or entirely prevented the development of an oxidized flavor in susceptible milk to which no copper had been added. Barnicoat and Palmer (2) studied the antioxidant properties of vitamins A, B, C, D, E, F, and nicotinic acid and found that only vitamins C and E were effective.

Gyorgy and Tomarelli (14) found corn, oats, wheat, rice, bran extract and Avenex to have high antioxidant properties. Of the known B vitamins, p-aminobenzoic acid was the only one they found to be significantly antioxygenic.

Tracy and Corbett (24) have shown that the addition of 0.1 to 0.25 per cent sodium citrate retarded the development of oxidized flavor in milk. Anderson (1) found that a pancreatic extract when added to milk protected it from developing an oxidized flavor.

The work of Dahle and Josephson (6, 7, 8), Corbett and Tracy (4), Koenig (17), Maack and Tracy (18), Mueller and Mack (20), and Peters and Musher (23) has shown that the use of oat flour or a water extract from the oat flour prevented or retarded the oxidized flavor development in milk, ice cream, frozen cream, and butter. Mueller and Mack (21) in studying the antioxidative properties of several cereal flours found that whole oat flour, finely milled oat flour, and corn flour were of equal value but that wheat, barley, rye, and rice flours had but little antioxidative effect.

Corbett and Tracy (5) reported the effectiveness of tyrosine, tyrosine esters and the water extract of certain cereal flours as antioxidants in dairy products. Hollender and Tracy (15) experimented with the use of certain antioxidants in powdered whole milk. Among the antioxidants they found most effective were gum guaiac, hydroquinone, ascorbic acid and sodium citrate.

The studies of McFarlane of MacDonald College, Quebec, as reported by correspondence with the author, have shown wheat germ oil to have antioxi-

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dant properties when added to certain animal and vegetable fats and oils used in the manufacture of shortening. The results were measured by means of the Swift's stability test. This investigator also found wheat germ oil particularly when fortified with citric acid retarded oxidative changes in powdered whole milk as measured by the peroxide value. McFarlane finds wheat germ oil to be more effective than either hydroquinone or gum guaiac.

Realizing the importance of copper contamination in the development of the oxidized flavor, modern dairies use stainless steel in the construction of their equipment wherever possible. In spite of this and other precautions that are taken to retain a normal flavor in the stored products, the industry still recognizes oxidized flavor development in dairy products as a serious problem. This is particularly true in the case of powdered whole milk, much of which is likely to be stored for some time at high temperatures (70° - 125° F.) before consumption. Because of the important part that powdered whole milk plays in the dietary of our armed forces as well as that of the civilian population of our allies, it seems that serious consideration should be given to the application of all known methods that will lengthen the shelf life of the product. Since any oxidation process to which the fat is subjected is likely to result in the destruction of certain of the vitamins contained in milk (Pavek and Shull (22)), it would also seem desirable from a nutritional point of view to add harmless antioxidants to those dairy products that are to be stored for some time before consumption.

EXPERIMENTAL DATA

The use of solvent extracted wheat germ oil as an antioxidant in milk, frozen cream, and powdered whole milk made by both the spray and roller methods has been studied. Representative results are given.

Use of wheat germ oil in pasteurized milk. An attempt was first made to determine to what extent wheat germ oil would retard the development of an oxidized flavor in unhomogenized whole milk. The wheat germ oil was added at three levels, 0.1, 0.2, and 0.3 per cent of the weight of the fat. The oil was mixed with the cold milk before pasteurization (143.5° F., 30 min.). The milk used was produced in late spring (May). To a portion of the milk, copper was added at the rate of 1 ppm. and 2 ppm. After 24 hours storage at 40° F. the samples were judged for flavor. The results are given in table 1.

A definite retardation of oxidation was evident in those samples containing the wheat germ oil and to which copper had been added. No particular benefits were obtained at levels higher than 0.2 per cent of the wheat germ oil. The best flavor was in the samples containing no added copper to which 0.1 per cent of wheat germ oil was added. At the 0.2 and 0.3 per cent level enough of the wheat germ oil flavor was noticeable to cause the milk to score less than the control.

TABLE 1

Wheat germ oil (A) as an antioxidant in whole milk

Sample	Flavor score* after 3 days' storage at 40° F.	
	Score	Criticism
Control	36	Sl. bitter
Control + 0.1% W.G.O.	37	Sl. oil flavor
Control + 0.2% W.G.O.	35	Sl. oil flavor
Control + 0.3% W.G.O.	34	Sl. oil flavor
Control + 1 ppm. Cu	29	Oxidized
Control + 1 ppm. Cu + .1% W.G.O.	31	Oxidized
Control + 1 ppm. Cu + .2% W.G.O.	32	Oxidized
Control + 1 ppm. Cu + .3% W.G.O.	32	Oxidized
Control + 2 ppm. Cu	28	Oxidized
Control + 2 ppm. Cu + .2% W.G.O.	31	Oxidized
Control + 2 ppm. Cu + .3% W.G.O.	31	Oxidized

* Basis of 45 as perfect score.

In other experiments it was found that at levels higher than 0.3 per cent of the weight of the fat the wheat germ oil flavor was sufficiently detectable in the milk to be objectionable even when copper was present.

Use of wheat germ oil in frozen cream. To study the effect of wheat germ oil in retarding the development of oxidized flavor in frozen cream, cream containing 50 per cent fat was mixed with sufficient sucrose to produce a sugar content of 12 per cent. Half of this sweetened cream was pasteurized at 160° F. for 30 minutes and the other half at 170° F. for 15 minutes. To half of each of the two lots 0.5 ppm. of copper was added. To a portion

TABLE 2

Wheat germ oil (A) as an antioxidant in frozen cream

Sample*	Degree of oxidized flavor after			
	4 mo.	6 mo.	9 mo.	13 mo.
A—Cream pasteurized at 160° F. for 30 minutes				
1A - Control	-	-	-	2½+
1B - Control	+	+	6+	7+
2A + 0.2% W.G.O.	-	-	-	-
2B + 0.2% W.G.O.	-	+	2+	4+
3A + 0.3% W.G.O.	-	-	-	-
3B + 0.3% W.G.O.	-	?	½+	3+
B—Cream pasteurized at 170° F. for 15 minutes				
1A	-	-	-	-
1B	-	-	+	5+
2A + 0.2% W.G.O.	-	-	-	-
2B + 0.2% W.G.O.	-	-	½-	2½+
3A + 0.3% W.G.O.	-	-	-	-
3B + 0.3% W.G.O.	-	-	?	2

* A = no copper added.

B = ½ ppm. Cu added.

of each of the four lots wheat germ oil at two levels (0.2 and 0.3 per cent of the weight of the fat) was added. The samples were stored in quart Seal-right containers in an ice cream hardening room and were judged every 3 to 4 months over a period of about 13 months. The results given in table 2 show that wheat germ oil caused a definite retardation of the oxidation of butterfat when copper was present. It is also evident that heating cream above 160° F. (170° F.) during pasteurization also aided in retarding oxidation. This confirms the results of Trout and Scheid (25) as well as Dahle, Lawhorn, and Barnhart (9), and McFarland and Burgwald (19). The beneficial effect of the higher heat treatment has been found by Gould and Sommer (11) and Josephson and Doan (16) to be due to the production of sulphydryls which are reducing substances. Best results were obtained when both the wheat germ oil and the high heat treatment were used. The superior effect of the combination was noticeable, however, only after nine months of storage.

Wheat germ oil as an antioxidant in powdered whole milk. Preliminary studies having established the antioxidant properties of wheat germ oil in milk and cream it was desired to determine to what extent the substance would function in powdered whole milk. A complete understanding of the nature of the ingredient contained in wheat germ oil that is responsible for the antioxidant properties is lacking. It is thought, however, that the tocopherols and phosphatides that are present are contributing agents. Since the start of this study it has been found that the potency of wheat germ oil can be increased by the addition of citric acid. The use of sodium citrate as an antioxidant in milk has been previously studied by Tracy and Corbett (24). Three different oils¹ were used, formula A—regular wheat germ oil, formula C—regular wheat germ oil plus 2 per cent citric acid, and formula D—regular wheat germ oil plus 5 per cent citric acid and 30 per cent soybean lecithin.

Procedure followed in preparing milk powder samples. Powdered whole milk prepared by both the vacuum roll and spray processes was used to test the antioxidant properties of the wheat germ oil. The milk used for the roller drying was produced by the University herd, while that spray dried was received at a commercial condensery. An attempt was made to handle the milk in such a manner as to minimize copper contamination.

The powder was packed in number 2 cans in the case of the roller process and number 1 cans in the case of the spray drier. Paper containers were used in one of the experiments with spray powder (samples 5019 and 5020—table 4). One set of the spray powders was packed plain and one set was packed in nitrogen.

The milk used to make the powder manufactured by the roller method (table 3) was preheated at 170° F. for 30 minutes and concentrated to ap-

¹ The wheat germ oil used in this study was solvent extracted and was supplied by the VioBin Corporation, Monticello, Illinois.

proximately 38 per cent total solids. Wheat germ oil—formulas A, C, and D, were added to the condensed milk at the rate of 0.2 per cent of the weight of the fat in trial 1 and 0.1 per cent in trial 2.

In the case of the spray dried samples 4924-31 and 4952-59 (table 4) the wheat germ oil was added to skim milk that had been heated to 170° F. and homogenized at 2,500 pounds pressure. This emulsion was then added to the raw milk and held over night. The milk was preheated at 180° F. for 7 minutes, cooled to 145° F. and then condensed. It was homogenized at 3,000 pounds at 130° F. The drying was then done 24 hours later. The nitrogen-packed powder was first subjected to a vacuum of 2 mm. Then CO₂ was introduced to 10 pounds pressure for 10 minutes, then a vacuum of 2 mm. was drawn, then nitrogen was introduced to a pressure of 5 pounds for 10 minutes.

TABLE 3

Use of wheat germ oil in roller (vacuum) process powdered whole milk

Sample	Trial	Flavor score		
		Fresh	3 mo.	6 mo.
Control	1*	38	29	29
Control + 0.2% W.G.O. A	1	38	33	32
Control + 0.2% W.G.O. C	1	38	34	32
Control + 0.2% W.G.O. D	1	38	34	33
Control	2†	38	37	33
Control + 0.1% W.G.O. A	2	38	36	35
Control + 0.1% W.G.O. C	2	38	37	35
Control + 0.1% W.G.O. D	2	38	37	36

* Prepared Nov., 1942.

† Prepared Feb., 1943.

Samples 4887-90 and 5017-22 were prepared in the same manner as those above except that nitrogen only was used in the case of the gas-treated lot.

The only variation in the procedure followed in the preparation of samples 4985-88 from that followed in the preparation of sample 4887-90 was the method of adding the wheat germ oil. Formula D was used in all samples. In the case of samples 4985 and 4986 the oil was homogenized in skim milk and added to the whole milk before condensing as outlined above. In the case of samples 4987 and 4988 the oil was mixed with the condensed milk just before spraying.

The dried milk samples were judged when fresh and every 2 to 3 months during storage at room temperature. In table 4 only the final flavor scores are reported. The flavor of the reconstituted samples was 38-39 at the beginning of the experiment and was criticized as being cooked. Subsequent scorings were made on the basis of the degree of oxidized flavor developed. The samples were reconstituted by mixing 30 grams of the powder with 200 ml. of tap water using an electric malted milk machine. The sample cans were opened immediately before the milk was reconstituted.

TABLE 4
Use of wheat germ oil in spray dried whole milk powder

Sample No.	Date made	Preheat temp. before condensing	Cone. of solids	Spray temp.	Spray pressure	Spray chamber temp.	Temp. of powder when packed	Type of pack*	Sol. in-dex	H ₂ O	Wheat germ oil used	Gas content†		Flavor score Aug. 7, 1943
												CO ₂	O ₂	
4924	11/24/42	180° F. 7 min., cooled to 145° F.	11.42 F.	115	2,000	170	106	P	0.1	2.1	None	3.64	4.24	30
4925	11/24/42		38.26 T.S.	115	2,000	170	106	N	0.1	2.1	None			32
4926	11/24/42		11.19 F.	115	2,100	170	106	P	0.1	2.3	0.1% A	3.81	3.5	31
4927	11/24/42		37.94 T.S.	115	2,100	170	106	N	0.1	2.3	0.1% A			33
4928	11/24/42		10.91 F.	115	2,300	170	102	P	0.1	2.0	0.1% C	4.17	3.15	31
4929	11/24/42	180° F. 5 min.	36.82 T.S.	115	2,300	170	102	N	0.1	2.0	0.1% C			33
4930	11/24/42		11.01 F.	115	2,400	170	102	P	0.1	2.0	0.1% D	3.21	3.75	31½
4931	11/24/42		37.41 T.S.	115	2,400	170	102	N	0.1	2.0	0.1% D			34
4952	12/2/43		11.44 F.	115	1,800	170	104	P	0.1	1.9	None	3.16	3.99	29
4953	12/2/43		38.64 T.S.	115	1,800	170	104	N	0.1	1.9	None			31
4954	12/2/43	170° F. 30 min.	11.28 F.	115	1,800	170	106	P	0.2	1.95	0.05% A	4.09	3.68	30
4955	12/2/43		36.93 T.S.	115	1,800	170	106	N	0.2	1.95	0.05% A			31½
4956	12/2/43		11.05 F.	115	1,800	170	106	P	0.1	1.66	0.05% C	3.25	3.65	30
4957	12/2/43		37.47 T.S.	115	1,800	170	106	N	0.1	1.6	0.05% C			31½
4958	12/2/43		10.98 F.	115	2,400	170	107	P	0.1	1.4	0.05% D	2.76	3.54	30
4959	12/2/43	170° F. 30 min.	36.50 T.S.	115	2,400	170	107	N	0.1	1.4	0.05% D			32
4887	11/10/42		11.59 F.	130	2,500	165	122	P	0.1	2.0	None	0.25	3.54	23
4888	11/10/42		37.60 T.S.	130	2,500	165	122	N	0.1	2.0	None			30
4889	11/10/42		11.47 F.	130	2,400	170	130	P	0.1	2.0	0.1% A	0.20	3.20	25
4890	11/10/42		37.53 T.S.	130	2,400	170	130	N	0.1	2.0	0.1% A			30
4985	12/12/42	180° 5 min., cooled to 145	10.90 F.	130	2,500	170	90	P	0.15	2.1	0.1% D	3.09	4.05	33
4986	12/12/42		38.56 T.S.	130	2,500	170	90	N	0.15	2.1	0.1% D			35
4987	12/12/42		10.87 F.	130	2,500	170	90	P	0.20	2.8	0.1% D	3.66	4.42	34
4988	12/12/42		38.35 T.S.	130	2,500	170	90	N	0.20	2.8	0.1% D			36
5017	1/13/43		10.06 F.	65	800	180	64	P-tin	0.10	1.8	None	0.28	1.45	32
5018	1/13/43	170° F. 30 min.	36.14 T.S.	65	800	180	64	N	0.10	1.8	None			35
5019	1/13/43			65	800	180	64	P-paper	0.10	1.8	None			33
5020	1/13/43		10.40 F.	65	800	180	63	P-tin	0.10	2.0	0.1%	0.22	1.75	34
5021	1/13/43		37.65 T.S.	65	800	180	63	N	0.10	2.0	0.1%			36
5022	1/13/43			65	800	180	63	P-paper	0.10	2.0	0.1%			36

* P=plain.

N= gas.

† Lots 5018 and 5021 were packed with water pumped nitrogen, while the other lots were packed with oil pumped nitrogen.

Other details regarding the procedure followed in making the spray powders are given in table 4.

Milk powder data. The data obtained on the effect of wheat germ oil upon the keeping quality of powdered whole milk are given in tables 3 and 4. It is evident that wheat germ oil retarded the oxidation of butter fat but did not prevent its occurrence. The oil treated with citric acid was slightly superior to the untreated oil. Formula D gave results somewhat better than those obtained with Formula C in most cases. The method of adding the oil was of no particular importance. The oil functioned in both plain and nitrogen-packed powder. It should be pointed out that neither the use of wheat germ oil or gas packing prevented oxidation from taking place though packing in the presence of an inert gas proved to be somewhat more beneficial than the use of the wheat germ oil without gassing. Best results were obtained with a combination of the two, however.

SUMMARY AND CONCLUSIONS

The ability of wheat germ oil to prevent oxidation has been determined in fluid milk, frozen cream and powdered whole milk made by both the vacuum roll and spray processes. The amount of wheat germ oil needed for best results is approximately 0.2 per cent of the weight of the fat. At higher levels (0.3 per cent) the flavor of the oil is sometimes detectable. Wheat germ oil reinforced with citric acid was found to be more effective in retarding oxidation in milk powder than regular oil. While wheat germ oil is not as effective as gas packing with nitrogen in preventing the development of the oxidized flavor in powdered milk, a combination of the two will prolong the shelf life of the powder more than either one alone will accomplish.

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TENTATIVE PROGRAM
AMERICAN DAIRY SCIENCE ASSOCIATION MEETING

TUESDAY, JUNE 20, 1944

Morning Session

9:00-12:00—General Meeting.

Welcome.

Response and presidential address—PRESIDENT A. C. DAHLBERG.

Address by guest speaker.

Afternoon Session

1:00- 4:00—Sectional Meetings—Extension Section, Manufacturing Section, and Production Section.

4:00- 5:00—Committee Meetings—Extension Section, Manufacturing Section, and Production Section.

Evening Session

Production Section Tentative Symposium.

WEDNESDAY, JUNE 21, 1944

Morning Session

9:00-11:00—Sectional Meetings—Extension Section, Manufacturing Section, and Production Section.

11:00-12:00—Joint Business Meeting of the Production Section and the Extension Section.

—Business Meeting of the Manufacturing Section.

Afternoon Session

1:00- 4:00—General Session—Post War Problems in Dairying.

Introductory Speaker.

From the Extension View Point.

From the Production View Point.

From the Manufacturing View Point.

4:00- 5:00—Committee Meetings—Extension Section, Manufacturing Section, and Production Section.

THURSDAY, JUNE 22, 1944

Morning Session

9:00-11:00—Sectional Meetings.

Manufacturing Section Symposium—Dehydrated Milk and Milk Products.

Joint Symposium—Production and Extension Sections.

- 9:00–9:15—Mastitis (from the dairyman's standpoint), T. S. SUTTON, Ohio State University.
- 9:15–9:45—Modern methods of treating mastitis, C. S. BRYAN, Michigan State College.
- 9:45–10:15—Discussion.
- 10:15–10:45—The feed situation, MR. WALTER BERGER, Chairman of the Feed and Livestock Division, Food Production Administration.
- 10:45–11:00—Discussion of feed situation.
- 11:00–12:00—Sectional Business Meetings—Extension Section, Manufacturing Section and Production Section.

Afternoon Session

- 1:00–3:30—Latin American Dairying.
WILLIAM S. HENDRIX, Ohio State University.
R. E. HODGSON, United States Department of Agriculture.
A. C. DAHLBERG, Cornell University.
- 3:30 —General Business Meeting.

Evening Session

- 6:30 —Annual Banquet.

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A STUDY OF CREAM RISING IN MILK

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The creaming process is a complex phenomenon that involves a number of factors. The difference in density between the fat and the milk plasma is the primary factor but a number of secondary factors, concerned with the formation of globule clusters, are required to make normal creaming possible. It has been shown that the individual globules rise in harmony with the velocity computed by Stokes' formula (4, 7, 8, 9), but this is too slow to account for the rapidity of normal creaming, the depth of the cream layer produced, and the sharp line of demarcation between the cream and skim-milk layers. The secondary factors, therefore, exert a controlling effect on the gravitational separation for creaming. The nature of these secondary factors and the detailed manner in which they function is still a matter of controversy.

The theory has been advanced that, aside from other factors, the gravitational movement of the globules (and clusters) is an important factor in cluster formation (1, 2, 5). The larger globules rising more rapidly overtake smaller globules, and as the clustering process proceeds in this manner, the clusters rise at an increasing rate and thus have increased opportunity for sweeping along individual globules as they occur in the upward path of the cluster. A difference in the fat content of the skim-milk at varied levels might well result from such a process. The study herein reported was undertaken to investigate this possibility.

EXPERIMENTAL

Samples of milk were placed in 1000-cc. graduated cylinders at 40° F. and stored in a refrigerator at that temperature. Six cylinders were used for each trial to provide for examination of a different cylinder at 1, 2, 4, 6, 10 and 24 hours after the cylinders were filled. Five experiments were conducted, one with fresh, raw milk and four with pasteurized milk. The

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milk had a fat content of 3.6 to 3.8 per cent. It was obtained from the mixed milk of about 40 herds as received commercially at the Department of Dairy Industry, University of Wisconsin. After the milk in the cylinders had stood undisturbed at 40° F. for the desired time, skimmilk samples were withdrawn at the following points as indicated by the graduated scale: 850, 750, 650, 550, 450, 350, 250, 150, 50 and 10 cc. The sample taken at the 850-cc. level was approximately 30 to 50 cc. below the cream line. The samples were withdrawn in the order as listed with the aid of the special apparatus shown in figure 1.

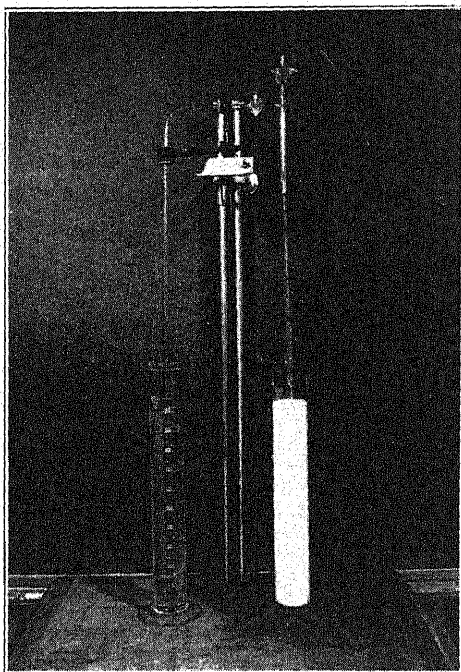


FIG. 1. Sampling device used for withdrawing samples of skimmilk at various levels from a graduated cylinder.

In the sampling procedure, entrance of the milk into the pipette was prevented until the tip was at the desired level. Mere closing of the mouth end of the pipette during lowering would not accomplish this since the hydrostatic pressure would compress the air and allow some milk to enter. This was counteracted to a point where a few small bubbles of air would actually escape from the pipette during immersion by simultaneously lowering a tube, connected to the pipette as shown in figure 1, into a sugar solution of sufficient density so that the hydrostatic pressure exceeded that caused by the milk. The assembly also carried a pointer, adjusted to the same level as the

pipette tip, to indicate its position as gauged by the pointer against the outside of the cylinder. When the pipette tip had been immersed to the desired level, the pipette was filled slowly with suitable manipulations of the valves and the application of suction. The entire assembly was then raised, the exterior of the pipette was wiped free from milk, and the sample within the pipette was then discharged into a suitable container. A clean, dry pipette was used for each sample. The fat content of the skimmilk samples was determined by the Mojonnier method as outlined by Mojonnier and Troy (3). All fat analyses were run in duplicate. The duplicate determinations

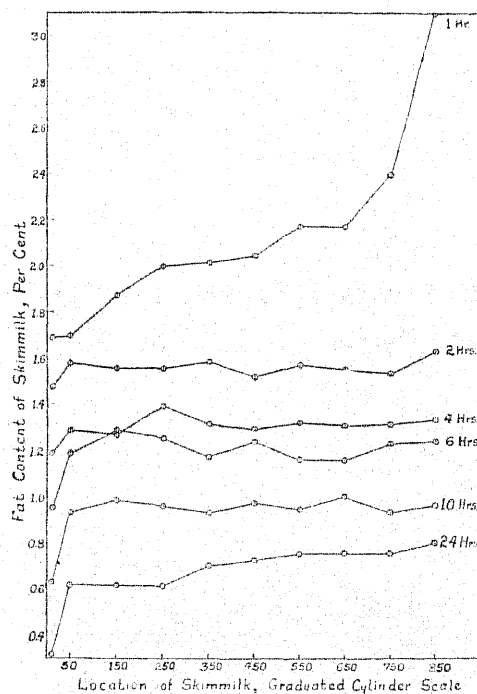


FIG. 2. Fat content of skimmilk at various levels below the cream line in 1000 cc. graduated cylinder used as a creaming vessel for whole pasteurized milk.

were made with considerable care and in general the difference between duplicates was very small in comparison with any observed differences in fat content between the various layers of skimmilk. Tables 1 to 5 give the duplicate fat tests and average fat tests of the skimmilk samples taken at various levels and at creaming times of 1 to 24 hours. The most rapid and extensive fat rising occurred during the first hour with a slow, gradual rise of the residual fat continuing during the succeeding 23 hours. While no data were secured beyond 24 hours, it can be assumed that a slow rising of residual fat would continue indefinitely in harmony with observations of Trelogan and Combs who studied rat rising in cream (6).

TABLE 1

Fat content of skim milk secured below the cream line in raw milk at varied levels

Skim milk level	Average and duplicate fat determinations			
	1 hour		2 hours	
		Average		Average
850	2.749		1.218	
	2.743	2.746	1.219	1.218
750	2.222		1.193	
	2.216	2.219	1.205	1.199
650	2.051		1.157	
	2.052	2.051	1.161	1.159
550	1.910		1.179	
	1.910	1.910	1.179	1.179
450	1.783		1.179	
	1.782	1.782	1.176	1.178
350	1.736		1.214	
	1.746	1.741	1.218	1.216
250	1.708		1.243	
	1.711	1.709	1.244	1.244
150	1.603		1.210	
	1.615	1.609	1.210	1.210
50	1.555		1.175	
	1.552	1.554	1.165	1.170
10	1.500		1.118	
	1.499	1.500	1.122	1.120
	4 hours		6 hours	
		Average		Average
850	1.111		0.887	
	1.113	1.112	0.893	0.890
750	1.123		0.906	
	1.108	1.115	0.896	0.901
650	1.125		0.900	
	1.121	1.123	0.901	0.900
550	1.140		0.902	
	1.143	1.142	0.906	0.904
450	1.123		0.900	
	1.117	1.120	0.898	0.899
350	1.129		0.898	
	1.121	1.125	0.896	0.897
250	1.166		0.881	
	1.167	1.166	0.880	0.880
150	1.151		0.896	
	1.149	1.150	0.887	0.892
50	1.136		0.858	
	1.136	1.136	0.865	0.862
10	1.037		0.789	
	1.045	1.041	0.796	0.782

TABLE 2

Fat content of skim milk secured below the cream line in pasteurized milk at varied levels

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	3.100		1.627		1.338	
	3.110	3.105	1.631	1.629	1.337	1.338
750	2.397		1.535		1.323	
	2.393	2.395	1.535	1.535	1.303	1.313
650	2.168		1.552		1.307	
	2.170	2.169	1.552	1.552	1.305	1.306
550	2.174		1.572		1.324	
	2.168	2.171	1.561	1.567	1.318	1.321
450	2.044		1.527		1.289	
	2.042	2.043	1.509	1.518	1.289	1.289
350	2.012		1.581		1.312	
	2.012	2.012	1.580	1.580	1.314	1.313
250	1.999		1.543		1.379	
	1.974	1.987	1.555	1.549	1.397	1.388
150	1.873		1.547		1.268	
	1.865	1.869	1.551	1.549	1.263	1.265
50	1.693		1.576		1.282	
	1.690	1.692	1.578	1.577	1.282	1.282
10	1.685		1.472		1.181	
	1.685	1.685	1.479	1.476	1.188	1.184
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	1.240		0.966		0.802	
	1.242	1.241	0.961	0.964	0.801	0.802
750	1.227		0.934		0.757	
	1.227	1.227	0.935	0.935	0.757	0.757
650	1.162		0.997		0.755	
	1.153	1.158	1.001	0.999	0.751	0.753
550	1.161		0.944		0.752	
	1.160	1.160	0.943	0.944	0.754	0.753
450	1.236		0.969		0.730	
	1.237	1.237	0.972	0.970	0.720	0.725
350	1.179		0.932		0.702	
	1.163	1.171	0.930	0.931	0.698	0.700
250	1.252		0.959		0.613	
	1.250	1.251	0.958	0.958	0.613	0.613
150	1.284		0.984		0.613	
	1.283	1.284	0.983	0.984	0.617	0.615
50	1.180		0.931		0.620	
	1.185	1.182	0.932	0.932	0.617	0.618
10	0.956		0.628		0.321	
	0.952	0.954	0.628	0.628	0.307	0.314

TABLE 3
*Fat content of skim milk secured below the cream line in pasteurized milk
 at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	2.711 2.705	2.708	1.709 1.696	1.703	1.368 1.362	1.365
750	2.420 2.408		1.696 1.697		1.293 1.305	
650	2.325 2.327	2.326	1.525 1.533	1.529	1.292 1.280	1.286
550	2.225 2.215		1.540 1.560		1.277 1.257	
450	1.997 1.986	1.992	1.515 1.524	1.520	1.255 1.253	1.254
350	1.969 1.971		1.527 1.531		1.246 1.240	
250	1.925 1.925	1.925	1.562 1.551	1.557	1.327 1.330	1.328
150	1.839 1.852		1.531 1.531		1.277 1.276	
50	1.823 1.815	1.819	1.510 1.506	1.508	1.337 1.322	1.330
10	1.620 1.615		1.399 1.410		0.994 0.994	
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	1.175 1.181	1.178	1.058 1.059	1.059	0.819 0.812	0.816
750	1.132 1.130		1.023 1.016		0.773 0.773	
650	1.161 1.161	1.161	1.013 1.013	1.013	0.763 0.761	0.762
550	1.105 1.127		0.964 0.974		0.739 0.728	
450	1.108 1.100	1.104	1.025 1.019	1.022	0.737 0.733	0.735
350	1.163 1.161		1.018 1.018		0.752 0.755	
250	1.159 1.164	1.162	1.004 1.002	1.003	0.729 0.728	0.728
150	1.165 1.155		0.971 0.979		0.731 0.739	
50	1.219 1.211	1.215	0.965 0.952	0.958	0.716 0.715	0.716
10	0.940 0.947		0.907 0.906		0.360 0.376	

TABLE 4

*Fat content of skimmilk secured below the cream line in pasteurized milk
at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	1.825		1.343		1.125	
	1.829	1.827	1.341	1.342	1.128	1.126
750	1.752		1.304		1.114	
	1.740	1.746	1.313	1.308	1.107	1.110
650	1.745		1.321		1.102	
	1.753	1.749	1.315	1.318	1.101	1.102
550	1.715		1.285		1.061	
	1.708	1.712	1.283	1.284	1.070	1.066
450	1.629		1.300		1.011	
	1.646	1.638	1.293	1.296	1.064	1.062
350	1.620		1.318		1.045	
	1.618	1.619	1.331	1.324	1.052	1.048
250	1.360		1.283		1.062	
	1.576	1.568	1.291	1.287	1.069	1.066
150	1.501		1.260		1.077	
	1.499	1.500	1.271	1.266	1.083	1.080
50	1.438		1.233		1.080	
	1.444	1.441	1.236	1.234	1.070	1.075
10	1.347		1.170		0.988	
	1.352	1.350	1.182	1.176	0.981	0.984
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	0.969		0.919		0.654	
	0.965	0.967	0.912	0.916	0.648	0.651
750	0.956		0.906		0.610	
	0.956	0.956	0.909	0.908	0.610	0.610
650	0.942		0.917		0.608	
	0.949	0.946	0.911	0.914	0.614	0.611
550	0.937		0.893		0.589	
	0.942	0.940	0.890	0.892	0.585	0.587
450	0.948		0.820		0.540	
	0.947	0.948	0.819	0.820	0.544	0.542
350	0.930		0.788		0.515	
	0.926	0.928	0.789	0.789	0.525	0.520
250	0.926		0.775		0.545	
	0.928	0.927	0.788	0.782	0.539	0.542
150	0.923		0.777		0.575	
	0.920	0.922	0.767	0.772	0.595	0.580
50	0.922		0.734		0.546	
	0.920	0.921	0.730	0.732	0.544	0.545
10	0.897		0.585		0.408	
	0.894	0.896	0.595	0.590	0.416	0.412

TABLE 5
*Fat content of skim milk secured below the cream line in pasteurized milk
 at varied levels*

Skim milk level	Average and duplicate fat determinations					
	1 hour		2 hours		4 hours	
		Average		Average		Average
850	1.643 1.645	1.644	1.341 1.346	1.344	1.114 1.123	1.118
750	1.561 1.565	1.563	1.278 1.285	1.282	1.093 1.103	1.098
650	1.543 1.537	1.540	1.281 1.290	1.286	1.150 1.154	1.152
550	1.586 1.587	1.584	1.272 1.286	1.279	1.108 1.103	1.106
450	1.575 1.583	1.579	1.259 1.264	1.262	1.136 1.142	1.139
350	1.574 1.578	1.576	1.280 1.270	1.275	1.103 1.106	1.104
250	1.516 1.513	1.514	1.281 1.283	1.282	1.113 1.120	1.116
150	1.488 1.474	1.481	1.278 1.289	1.284	1.135 1.132	1.134
50	1.446 1.452	1.449	1.272 1.274	1.273	1.112 1.111	1.112
10	1.345 1.339	1.342	1.237 1.237	1.237	0.964 0.959	0.962
	6 hours		10 hours		24 hours	
		Average		Average		Average
850	0.950 0.946	0.948	0.929 0.935	0.932	0.603 0.602	0.602
750	0.967 0.963	0.965	0.860 0.856	0.858	0.657 0.608	0.662
650	0.968 0.972	0.970	0.855 0.862	0.858	0.599 0.600	0.600
550	0.964 0.964	0.964	0.871 0.877	0.874	0.608 0.618	0.613
450	0.973 0.971	0.972	0.878 0.874	0.876	0.621 0.617	0.619
350	0.989 0.980	0.984	0.867 0.874	0.870	0.610 0.616	0.613
250	1.006 1.008	1.007	0.870 0.867	0.868	0.592 0.604	0.598
150	0.967 0.960	0.964	0.890 0.897	0.894	0.587 0.590	0.588
50	0.966 0.966	0.966	0.865 0.861	0.863	0.601 0.607	0.604
10	0.870 0.868	0.869	0.598 0.595	0.596	0.467 0.462	0.464

In the gravitational theory of clustering, which inspired this study, it had been anticipated that, at some stages in the creaming process, the fat content of the skimmilk at some of the higher levels might actually be less than that of skimmilk at a lower point. This anticipation is on the basis of the greater sweeping action to which the fat globules in the upper portions of the skimmilk are subjected. The data indicate that such differences occur to a slight degree, but surprisingly the data suggest further that there is a wave-like progression of fat in the creaming process. This trend is revealed in the most pronounced manner by the data given in table 2 which have been presented graphically in figure 2.

No explanation is offered for the wave-like progression of the fat in the trials herein reported. While this tendency is suggested, the present approach is not ideal for a complete verification of this point. In order to establish this conclusion definitely, it would obviously be necessary to obtain a large number of points so that each wave in the graph might be defined by several points. It would then be necessary to use a method of sampling which would assure as far as possible a perfect horizontal section of the column of skimmilk at the selected level. While the present equipment was intended to attain a sample of skimmilk at a precise level, its use in sampling at still closer intervals probably would not be justified because of the manner in which the milk flows into the pipette.

SUMMARY

It was shown that in the creaming of milk the greater portion of the fat rises during the first hour. A slow, gradual rise of residual fat continues indefinitely. There is some indication in the data that during the creaming process the fat content of the skimmilk at one level may be slightly less than the fat content of the skimmilk at a lower level. Indications were obtained that such differences may occur at several points in a skimmilk column suggesting a wave-like progression of the fat. This suggestion should be further verified before it is accepted as a definite conclusion. There appears to be no difference in the manner in which the cream layer forms on raw milk as compared with pasteurized milk.

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EFFECT OF ADDING COD LIVER OIL TO THE RATIONS OF DAIRY CALVES*

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Some calves dropped in the dairy herd at University Farm are weak at birth, and develop indigestion or other ailments and a few die before they are a month old. The cows in the herd are fed largely on a barn-feeding basis throughout the entire year because little or no pasture is available. Fair to good quality roughage is fed. Meigs and Converse (6) have shown that the quality of roughage fed to cows has a marked effect on the health and vigor of their calves at birth. Similarly the beneficial effect of feeding a vitamin A supplement to calves for several weeks after birth or until they are consuming considerable quantities of hay has been demonstrated by Phillips (7). If carotene-poor roughage is fed, or if young calves, for any reason, fail to utilize the carotene supplied then the feeding of a vitamin A-rich product beyond the first few weeks should prove beneficial. Ellenberger (4) reported that neither calves raised to 240 days of age on poor quality roughage nor those fed good hay for the same period evidenced any lack of vitamin A. Calves fed the poor hay, however, made "slower, lesser, and more costly growth" as caused by their lower consumption of nutrients in the less palatable ration. When good quality hay was fed, no benefits appeared from feeding cod liver oil, instead growth in some cases seemed to be retarded. Cary (2) states that calves fed skim milk, grain and hay after 30 to 60 days of age must, in order to survive, receive a vitamin A supplement if fed hay as poor in carotene as U. S. No. 3 alfalfa or timothy. Feeding at the rate of 20 milliliters of cod liver oil per calf daily to six months of age, however, had no demonstrable effect on their general appearance and rate of growth when U. S. No. 2 alfalfa hay or better was fed. Similarly, Insko and Rupel (5) observed no marked difference in the rate of growth and composition of bones between calves fed a standard ration, including good quality legume hay, and others reared on a similar ration supplemented with cod liver oil. Similar results have more recently been reported by Reaves and Cannon (8). In a comparison involving four pairs of calves from dams that presumably obtained a normal allowance of pasture, Dahlberg and Maynard (3) fed varying amounts of Nopco XX, a vitamin A and D rich concentrate of cod liver oil. Both lots weighed exactly the same at the start, but the supplemented group soon forged ahead and at 26 weeks of age each averaged 18.7 pounds more than those in the check group. The

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authors concluded that if there was any difference in general health it was in favor of the calves receiving the supplement.

EXPERIMENTAL

The object of the experiment reported here was to determine the effect, if any, on the growth and well-being of dairy heifer calves at University Farm of supplementing their rations with cod liver oil from birth to six

TABLE 1

Effect on the weight of calves of various breeds of including cod liver oil in their rations to 180 days of age. Data calculated to a 10-day interval basis

Age	Guernseys		Holsteins		Jerseys	
	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil
	No. in group					
	7	12	14	14	12	13
<i>days</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Birth	64.0	65.9	94.7	93.6	53.9	53.2
10	66.4	67.2	101.2	97.9	55.8	56.5
20	70.6	72.4	112.7	108.9	62.2	63.3
30	80.5	80.2	126.1	121.8	68.5	72.1
40	86.4	88.3	137.0	138.4	76.8	82.4
50	97.5	94.6	152.6	152.6	90.4	90.0
60	104.4	102.2	171.0	169.6	101.6	101.2
70	114.6	114.4	187.1	185.0	112.6	113.8
80	126.7	127.8	201.8	201.7	122.4	127.2
90	136.1	142.6	219.7	217.3	134.1	141.5
100	149.6	157.4	235.7	235.2	148.5	157.4
110	162.7	171.9	256.0	251.9	159.3	170.2
120	174.9	188.9	274.8	271.8	173.7	186.0
130	189.4	204.5	292.6	290.4	185.5	197.0
140	204.6	222.5	309.5	307.5	201.5	211.2
150	217.2	239.3	329.1	326.2	216.3	226.6
160	235.5	255.1	347.6	350.2	232.9	244.2
170	250.8	270.8	367.3	370.0	247.3	261.1
180	262.5	286.9	389.1	389.0	258.7	275.5
Total gain in 180 days	198.5	221.0	294.4	295.4	204.8	222.3
Average daily gain	1.10	1.23	1.64	1.64	1.14	1.24

months of age. The trials were carried on with all the heifer calves born in the herd during a period of about three years. It included 19 Guernseys, 25 Jerseys and 28 Holsteins. The calves were assigned at birth alternately to the check and experimental groups. The calves in both groups were fed and handled alike except that 25 to 35 cc. of cod liver oil (U.S.P.) was fed daily to each animal in the supplemented group. Whole milk was fed the first 30 days, and then skim milk to six months of age. The milk was fed at the rate of one-eighth the weight of the animal but not over 16 pounds daily per calf. Calves were allowed to eat all the alfalfa hay they wanted

at all times. Fair to good quality hay was fed. A grain mixture consisting of equal parts by weight of oats, corn, and barley (all ground medium fine), and wheat bran was fed *ad libitum* up to four pounds daily per calf. Water and salt was available to the calves at all times. Calves were kept in large roomy pens with four or five of the same breed and of approximately the same age in each. They were allowed to exercise outdoors several hours daily when weather conditions permitted.

Each calf was weighed within a few hours after birth and at regular ten-day intervals thereafter. Measurements of height at withers were made every 30 days beginning within a few weeks after birth. Frequent observations were made of the physical condition and appearance of each calf.

TABLE 2

Effect on the height at withers of calves of various breeds of including cod liver oil in their rations to 180 days of age. Data calculated to a 30-day interval basis

Age	Guernseys		Holsteins		Jerseys	
	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil	Non-cod liver oil	Cod liver oil
	No. in group					
	7	12	14	14	12	13
<i>days</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
10	70.4	71.8	74.8	74.1	67.3	67.9
30	72.4	73.5	77.6	78.1	69.4	69.8
60	76.6	76.8	83.4	83.6	75.1	75.0
90	81.2	81.2	89.4	88.8	80.1	80.8
120	86.0	86.6	94.2	94.0	84.8	86.3
150	90.4	91.8	99.2	98.6	89.8	90.5
180	94.7	96.2	104.2	103.1	93.4	95.0
Total gain in height in 170 days	24.3	24.4	29.4	29.0	26.1	27.1
Average daily gain	0.14	0.14	0.17	0.17	0.15	0.16

RESULTS AND DISCUSSION

Data are presented in table 1, showing the weights at ten-day intervals, from birth to 180 days of age, of groups of calves of the Guernsey, Holstein, and Jersey breeds respectively, when fed cod liver oil and of corresponding non-supplemented groups. Data relating to the average height at withers of the animals in the various groups at 30-day intervals are presented in table 2. As height measurements of calves at birth were seldom made, none are given in the table.

From the data in tables 1 and 2 it is apparent that adding cod liver oil to the rations had no evident effect on the rate of gain in height at withers of calves of any breed nor on the gain in weight of Holsteins. On the other hand the Guernseys and the Jerseys fed cod liver oil, gained an average of

22.5 pounds and 17.5 pounds more respectively per calf during the 180 days than those in corresponding non-supplemented groups. Statistical analysis of the data indicate that these differences are slightly significant (9).

The recorded observations indicate that less trouble from digestive disturbance was encountered in young calves that were fed cod liver oil than among those in the non-supplemented groups. It is also significant that a few calves in the non-cod liver oil groups died within a few weeks after birth, and others apparently were saved from this fate when cod liver oil was added to their rations. These facts largely account for the difference in number of calves in the supplemented and the non-supplemented groups. Data relating to the calves that died during the experimental period or that were transferred to a supplemented ration are not included in tables 1 and 2.

Contrary to what may have been expected, as based on the studies of Phillips (6) the cod liver oil supplement had no apparent effect on the rate of gain of calves until after they were about 90 days old. It seems probable that the whole milk, which, generally is a rich source of vitamin A and carotene, helped to build up a small reserve of these factors in the body of the calf during the first 30 days when it was fed and this together with the carotene obtained in the limited quantity of hay consumed provided sufficient vitamin A for growth to about 90 days of age. Beyond that age, however, with the vitamin A reserve of the body largely exhausted the supply obtained from the roughage in the ration was not fully adequate except in the case of the Holstein calves. This difference may have been due to a higher carotene requirement by the Guernsey and the Jersey calves as has been suggested by Boyer *et al.* (1), or to their inability to utilize this factor as efficiently as the Holsteins. Also, it is possible that the Holstein calves consumed proportionately more roughage and thus obtained correspondingly more carotene. Unfortunately complete records were not kept of the quantity of hay consumed by each calf. To what extent results would have been changed had excellent, instead of fair to good, quality hay been fed can only be surmised. From the data presented it appears that it may be beneficial to feed cod liver oil to young calves, especially Guernseys and Jerseys, when roughage only fair in quality is fed.

SUMMARY

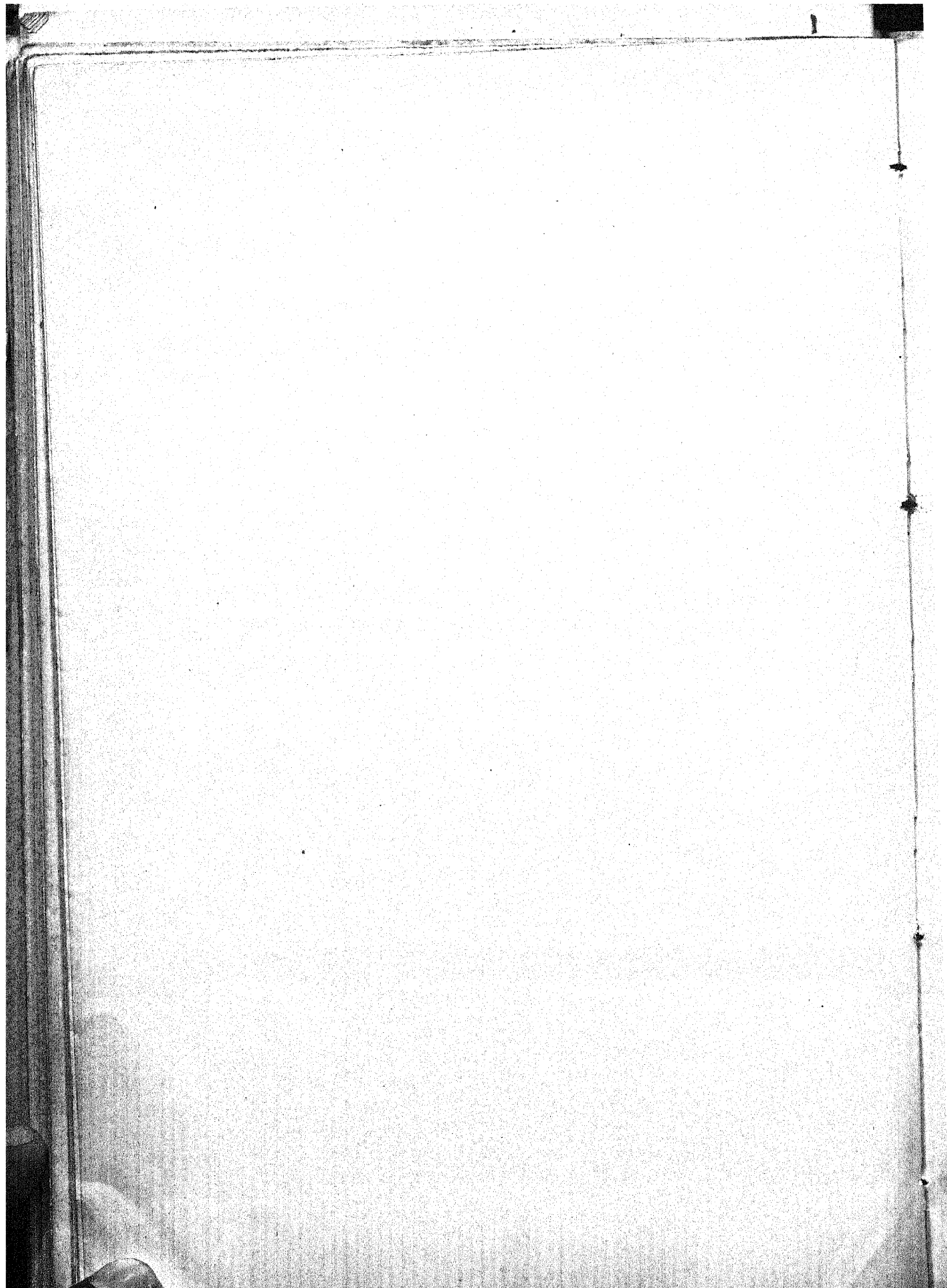
Comparisons were made using a total of 72 calves, including 19 Guernseys, 28 Holsteins and 25 Jerseys, to determine the effect, if any, of adding cod liver oil, as a vitamin A supplement to the rations commonly fed to calves during the period to six months of age. Alternate animals were fed 25-35 cc. cod liver oil daily beginning when the calf was only a few days old, in all other respects the plan of feeding was alike for both groups.

Somewhat less digestive troubles occurred in the calves fed cod liver oil than among those in the non-supplemented groups. No significant differ-

ence was noted in any breed in rate of gain in height at withers between the calves fed cod liver oil and those in the check group. Adding cod liver oil to the ration had no evident effect on the rate of gain in weight of Holstein calves. The Guernsey and the Jersey calves fed cod liver oil, on the other hand, gained an average of 22.5 pounds and 17.5 pounds more per calf respectively during the 180-day period than those fed similar but non-supplemented rations.

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THE RELATION OF CHLORINE AND CATALASE CONTENT OF MILK TO ITS CURD TENSION*

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The type of curd obtained from milk with rennet has been studied by many in attempts to relate the nature of the curd to one or more of the properties of the milk. The reaction of the enzyme and the milk will vary from sample to sample in its speed, that is, in the time required for the rennet to cause visible curdling under standard conditions of temperature and concentration of enzyme. The physical properties of the coagulum will also vary from sample to sample. Most observations have been limited to the product of individual cows and also to relatively short periods. A field project on the manufacture of cheese presented an opportunity to study curdling properties and milk composition of each of six herds daily throughout a lactation period and the milk of each animal in each herd several times in the period.

The animals of the six herds, totaling 157 milking cows, freshened in early spring and were dried off in early November. The resting time between lactation periods was thus longer than in herds in which production is maintained at a relatively constant level throughout the year.

Firmness of the curd produced under standard conditions of temperature and concentration of rennet, chlorine and catalase content of the sample, and also the reducing power of each sample toward methylene blue and toward resazurin were observed. The accumulated experience of many has shown that the mass of reproducing bacteria of the milk is the chief reducing constituent of milk toward methylene blue and that the mass of growing bacteria and the mass of body cells in milk are the reducing agents toward resazurin. The action of each of these two agents on resazurin can be separately determined in some degree when the reducing power of the milk on this reagent is compared with the action on methylene blue. The reduction of resazurin can be divided into two phases: first, a change from the original slate gray color of the milk-resazurin mixture to a pink, and second, a change of the latter to white. The extent of the progress in each phase can be observed. If the content of a milk in body cells (leucocytes) or bacteria is high, the time required for the first phase of the reduction of resazurin will be short. The answer as to which of the reducing agents is responsible for a color change noted with resazurin is supplied by comparing results of the two

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tests. If the bacteria are the chief cause of reduction in any sample, reduction times with the two dyes will be much the same. If, however, the methylene blue indicates presence of few bacteria, then the rapid reduction of resazurin to its pink form is due to body cells, the presence of which is indicated by the high catalase content, since the body cells are apparently the source of the catalase of milk. Its other constituents are devoid of this enzyme. The chlorine content of milk high in catalase will also be high since blood serum will also be entering the milk system if cells are passing from the blood system into the milk system. Thus chlorine, catalase and body cells will tend to vary together in the milk of an individual cow and these variations will cause variations in reducing power of the milk toward resazurin. High bacterial content will tend to obscure the latter relation.

The entrance of blood serum into the milk system tends to raise the pH of the milk and increase the sodium: calcium ratio, changes that alter the

TABLE 1
The season's history of the herds studied

Herd	May 9				Sept. 30				Oct. 24			
	Cows		Milk		Cows		Milk		Cows		Milk	
	No.	%	lbs.	%	No.	%	lbs.	%	No.	%	lbs.	%
1	34	100	1120	100	24	70	576	60	20	60	364	32
2	33	100	902	100	31	94	446	49	31	91	324	36
3	27	100	791	100	27	100	569	72	25	92	387	49
4	21	100	450	100	17	81	235	52	14	66	242	53
5	24	100	576	100	21	88	497	86	21	88	314	54
6	18	100	606	100	17	94	360	57	15	83	258	42
Average	157	100	741	100	137	87	447	60	126	80	315	42

speed of reaction of milk with rennet and also the properties of the coagulum. It seems probable that the presence of an inflammatory process in the udder causes a decrease in the percentage of casein from that normal for the animal and changes its quality in such a manner that curdling with rennet is retarded and the firmness of the curd is decreased from that normal for the animal. It is also to be remembered that the curds formed in the milk of different cows by rennet will vary in physical properties due to the genetic pattern of the animal. In this case the four quarters of the udder should produce milk showing the same type of coagulum, while in the case of an inflammatory process the nature of the coagulum noted in the product of the affected quarter or quarters would differ from that of the normal quarter.

This study is one of the relation of mastitis to the quality of milk and to the quality of the cheese made therefrom. The discussion is based wholly on comparative values, so it does not seem essential to describe in detail the methods used. The property of the curd observed was firmness or tension.

The seasonal record of the herds is presented in table 1.

The curd tension of the entire quantity of milk produced in 24 hours was noted once or twice each week throughout the season. Readings were made 30 and 40 minutes after the rennet was added to the milk. The relative values were the same in either case. Only those noted after 30 minutes are given in table 2. The product of herd 4 is markedly different in curd tension from that of the other herds.

In the detailed study of each herd it seemed that the clearest picture of the relation of mastitis to curd tension and to other items of interest would be obtained by comparing animals that in May produced milk of a curd tension of 5 or below with animals that during the same period produced milk having a curd tension of 40 or above. One point of interest is the relation of curd tension to persistence in production. The data are presented in table 3. The significance of the data is only indicative on account of the limited number of animals in each herd.

TABLE 2
Average curd tension by months of herd milk

Herd	1	2	3	4	5	6
May	13	17	18	22	12	14
June	9	9	6	13	3	4
July	10	12	5	14	4	8
August	4	6	5	11	2	4
September	5	7	4	16	2	5
October	12	14	12	20	10	9
Average	8.8	10.8	8.3	16.0	5.5	7.3

The entire comparison may be thus expressed. Twenty-five cows that produced milk of a curd tension of 5 or below in May gave in late September 53.1 per cent as much milk as in May. Twenty-four cows that in May produced milk with a curd tension of 40 or above gave in September 65.0 per cent as much milk as in May. The observations conform with what one would expect in showing that mastitis is a factor in persistence of production in any lactation period.

CURD TENSION IN RELATION TO CHLORINE AND CATALASE CONTENT

The relationship of curd tension to chlorine and catalase content of milk was also studied, using only the extreme cases, *i.e.*, those with a curd tension of 5 or below and those with a curd tension of 40 or above. The judgment as to the members of the intermediate group would be questionable, hence the use of the extremes rather than the entire number of cows in the six herds.

The catalase content of the milks was determined by collecting the total amount of oxygen set free from an excess of hydrogen peroxide by a quantity of the milk and expressing it in terms of percentage of the quantity of milk

TABLE 3
The relationship of curd tension to persistence in production

	Curd tension	On Sept. 25 produced of the May 8 yield
Herd 1		<i>Per cent</i>
3 cows	5 or less	24.7
3 cows	40 or more	68.0
Herd 2		
9 cows	5 or less	42
3 cows	40 or more	50.0
Herd 3		
5 cows	5 or less	74
3 cows	40 or more	60
Herd 4		
0 cows	5 or less
6 cows	40 or more	65
Herd 5		
7 cows	5 or less	92
2 cows	40 or more	100
Herd 6		
1 cow	5 or less	33
7 cows	40 or more	48

used, thus if 10 cc. of milk set free 10 cc. of oxygen its catalase value would be 100.

The values given in table 4 are the average of all observations during the entire period of observation. The relation of high chlorine and catalase content to low curd tension is to be noted in each herd except herd 5. Since the record of this herd differs so markedly from that of the other five herds, the detailed data are presented in table 5. Herd 5 contained many cows producing milk of low curd tension with no evidence of udder trouble as shown by the chlorine and catalase content of the milk. It has long been known that some cows normally produce soft-curd milk, while in others the changed composition of the milk is due to a disturbance in the physiology of the

TABLE 4
The catalase and chlorine content of herd milk of low and high curd tension in May

Herd	Curd tension			
	5 or less		40 or more	
	Chlorine	Catalase	Chlorine	Catalase
	%	%	%	%
1	0.169	145	0.117	34
2	0.170	97	0.125	38
3	0.146	52	0.128	15
4	0.117	26
5	0.114	18	0.130	10
6	0.184	59	0.121	22

udder. As far as the writers know, this is the first instance of a herd of apparently normal cows producing milk of low curd tension or soft-curd milk. The herds studied consist largely, if not wholly, of their own progeny; the genetic factor responsible for soft-curd milk may thus be brought to attention in a degree not probable in a herd maintained by purchase.

TABLE 5

The catalase and chlorine content of the milk of individual cows of low and high curd tension in herd 5

Cow	Curd tension	Chlorine	Catalase	Cow	Curd tension	Chlorine	Catalase
May 9, 1941							
23	0	0.114	17	1	63	0.135	10
				5	48	0.122	14
				12	42	0.133	6
May 26, 1941							
6	0	0.085	7	2	48	0.088	2
13	0	0.102	15				
14	0	0.091	4				
15	0	0.097	28				
18	0	0.091	4				
19	0	0.093	17				
22	0	0.101	5				
23	0	0.083	10				
25	0	0.092	6				
27	0	0.089	35				
July 21, 1941							
6	0	0.082	12	1	42	0.087	17
7	0	0.100	3	2	52	0.082	2
14	0	0.102	6	4	65	0.101	...
15	0	0.079	50	5	43	0.086	5
18	0	0.103	10	11	40	0.098	14
22	2	0.103	9				
23	0	0.099	7				
27	5	18				
				17	44	0.103	9
				24	43	0.103	33
Aug. 7, 1941							
6	0	0.079	21	2	65	0.081	5
7	0	0.066	10	12	52	0.062	14
14	0	0.086	18	17	50	0.097	13
15	0	0.100	69	24	42	0.106	36
18	0	0.107	17				
22	0	0.087	39				
23	0	0.084	30				
27	4	0.090	47				

The resazurin tests were observed at the end of each hour for five hours, and a number given to each sample at each observation. A low number, 1, for example, indicates that little change in the color of the dye was noticed

TABLE 6

The relationship of resazurin reduction after 1 hour incubation to curd tension, chlorine and catalase content of milk

Herd	Curd tension	Cows	Chlorine	Catalase	Resazurin
1	5 or less	6	0.169	145	4.0
1	40 or more	10	0.117	34	2.0
2	5 or less	15	0.170	97	3.5
2	40 or more	3	0.125	38	1.0
3	5 or less	6	0.146	52	1.6
3	40 or more	3	0.128	15	1.0
4	5 or less	0
4	40 or more	7	0.117	26	1.5
5	5 or less	1	0.114	17	1.0
5	40 or more	3	0.130	10	1.3
6	5 or less	2	0.184	59	4.0
6	40 or more	6	0.121	22	1.5

at that period of observation, while a high number indicated much greater reduction at the period of observation. Thus, 1 at the fourth hour means a milk very low in bacterial content and in cells, while 5 at the first hour means a milk high in bacteria or in body cells, or in both. The answer as to which is given by the methylene blue test. The milks in question were constantly so low in bacteria that a rapid reduction with resazurin may be used as an evidence of high content in body cells. It thus seems proper to use the averages of the resazurin reading of the two classes of cows—those producing milk of a curd tension of 5 or below and those showing a tension of 40 or above.

Table 6 presents the average reading of the resazurin test at the end of the first hour for the two groups of cows in each of the six herds on a particular day in May. The expected relation between high chlorine and catalase content and rapid reduction of resazurin is shown in each herd except 5, in which the more rapid reduction was noted in the high tension group of cows. The number of cows in each group of this herd was so small that the figures can be only suggestive. The smallness of the groups representing the extreme high and low values indicates that the herd consists largely of normal animals.

TABLE 7

The relationship of catalase content of milk to the rapidity of reduction of resazurin

Herd	Average catalase content	Average reading in resazurin test, 1 hr.
1	56	3.0
2	65	3.2
3	48	2.5
4	29	1.9
5	25	1.4
6	48	2.7

The relationship between the average catalase content of the entire amount of milk delivered at the factory to the average reading of the resazurin test at the end of the first hour for each herd is shown in table 7.

The relationship between catalase content and reducing power toward resazurin is clearly evident from the data, which also show that herd 5 is the only one of the six that can be considered as producing normal milk, since the milk of cows with healthy udders should not exceed 25 in its catalase value. Indeed, the value 20 is most frequently found in the literature on the subject, and in the experience of the writers some herds will consistently produce milk that will not exceed this value. The same is true of most first lactation cows.

The cause of the condition noted in herds 1, 2, 3 and 6 cannot be stated with certainty. From the few observations made as to the presence of streptococci in the milk of individual cows, one is inclined to consider them as the prime disturbing agent. The observations of the senior author indicate that herds in similar condition are to be found in all areas. It is hoped that the incidence of such herds is not as high as in the six studied, for they cannot serve as the foundation of a successful farm or of a successful cheese industry. Some program of herd management should be developed through the use of which such herds can be placed on a more normal base.

SUMMARY

The tension of the curd produced under standard conditions in the 24-hour product of each of six herds was noted frequently throughout a period of six months. The product of one herd (No. 4) had an average curd tension approximately twice as great as that of the other five herds and is believed to represent what one would expect to obtain from a group of cows with healthy udders. The low curd tension of four of the remaining five herds is believed to be due to the high incidence of chronic mastitis in these herds, since the product thereof was abnormally high in catalase and in chlorine. The low curd tension noted in the product of the remaining herd (No. 5) is believed due to the genetic pattern of the members of the herd since the chlorine and catalase content of its product was normal.

The record of the four herds indicates them to be agents of low value for the production of milk both from the standpoint of quantity and quality of milk.



THE VISCOSITY OF EVAPORATED MILKS OF DIFFERENT SOLIDS CONCENTRATION

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The viscosity of evaporated milk is an important characteristic because, in the minds of many consumers, it indicates a quality of "richness." Of greater significance, however, is the fact that an increase in the viscosity of the evaporated milk retards the rate of fat-separation in storage, since the phenomenon of fat-globule-rise is largely a function of size of globules and viscosity of medium. Objectionable fat-separation may occur because of improper homogenization of the milk or because the body of the evaporated product is too "thin." This report is a discussion of a preliminary study of the viscosity of evaporated milks of different concentrations and of the effects of different manufacturing and storage conditions.

Some years ago preliminary observations were made in these laboratories on evaporated milks that contained approximately 26 per cent of milk solids. Forewarming the whole milks up to 100° C. (212° F.) was carried out in a steam-jacketed hotwell; forewarming at 100° C. to 120° C. (248° F.) was accomplished in an autoclave. The reciprocal relationship of heat stability and consistency was noted in many instances. For example, forewarming a milk at 70° C. (158° F.) for 10 minutes usually rendered its evaporated product less stable than an evaporated product from a milk heated to 95° C. (203° F.) for 10 minutes. However, as a rule the consistency of the former product at coagulation was greater. In several cases, milks heated to 105° C. (221° F.) for 10 minutes produced evaporated products of greater stability than those from other samples forewarmed at lower temperatures. The consistencies of the milks heated to the higher temperatures were less.

These results were similar to those obtained by Grindrod (1), who subjected milk to the "impact process" of sterilization and found that milk heated to 110° C. for one minute possessed greater heat stability than did milk forewarmed at the commercially used temperatures (90°-100° C.). The resulting evaporated milk was too fluid.

H. Ziker (2) states that forewarming temperatures above the boiling point produce maximum stability but low viscosity while the reverse is true at lower forewarming temperatures. Recent work in these laboratories (5, 6) has shown that the heat stability and concentration of evaporated milks can be greatly increased by the use of high temperatures applied to the milk for a short time. By means of this method of treatment, samples with a wide range of concentrations and stabilities were made available for viscosity determinations.

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EXPERIMENTAL

Whole milk from the Beltsville herd of the Bureau of Dairy Industry was standardized to a fat to solids-not-fat ratio of 1 to 2.29. Experimental samples were prepared, both with the usual forewarming and with the high-temperature forewarming procedure described elsewhere (5, 6). The milks forewarmed at 95° C. for 10 minutes were heated in a steam-jacketed hotwell. Those forewarmed by the high-temperature short-time method were heated in a Mallory heater (3).

After the milk was forewarmed it was concentrated in a stainless steel vacuum pan to 32-35 per cent solids, heated to 60° C., homogenized at a pressure of 2500 pounds per square inch, and cooled. Precautions were taken throughout the work to obtain uniform homogenization in all batches and thus to eliminate any variable which might result from a lack of uniformity in fat-globule size. Small quantities of the milk were diluted with water to the desired solids content. The heat stability, or time required to initiate coagulation, in these samples at 115° C. was then determined by heating them in small cans in a pilot sterilizer. The sterilizer reel revolved at the rate of 4 rpm. and during the treatment of all samples it was allowed to run continuously.

Viscosity determinations were made on 150-gram samples with a McMichael viscosimeter. The measurements were made at 20° C., unless otherwise stated. The wires were standardized against sugar solutions of known viscosity and all results were converted to centipoises. The cans of milk were stored in constant-temperature rooms, undisturbed, until viscosity determinations were to be made. Samples were adjusted to the temperature of measurement by immersing the cans in a water bath for several hours before opening them. Excessive fat-separation in occasional samples made it impossible to obtain accurate measurements on such milks, and consequently some inconsistencies in the data were caused by this change in fat-dispersion.

RESULTS

The data plotted in figures 1 and 2 show the course of viscosity development in concentrated milks of different types during heating to and beyond the point of coagulation. The effect of aging milk and of the addition of lactic acid upon the body produced during sterilization is shown in figure 1. The curves in figure 2 show the viscosities of milks of different concentrations after various periods of heating at 115° C. The samples of figure 1 were forewarmed by usual commercial methods (95° C. for 10 minutes) while those of figure 2 (except the "95°" controls) were forewarmed at high temperatures and held for 25 seconds. These figures indicate how rapidly the viscosity of the evaporated milks increased just before and after coagulation. The viscosities at coagulation decreased as the heat stabilities of the milks increased.

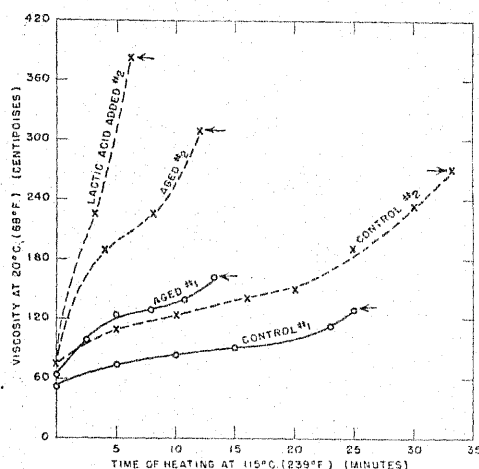


FIG. 1. The relationship between the heat stability of concentrated milk and the viscosity which is developed in it during heating at 115° C. (239° F.). The arrows indicate the time of the appearance of visible coagulation. All milks were forewarmed at 95° C. (203° F.) for 10 minutes before concentration to 26 per cent solids content.

The relationship between the viscosities of evaporated milks of different solids content and their heat stabilities is shown in figure 3. The body of the

TABLE 1

The effect of differences in heat stability upon the viscosity of evaporated milks sterilized at 115° C. (239° F.) for 18 minutes and held in storage. The different stabilizing heat treatments used on the raw and concentrated milks are indicated. Samples bearing the same date were prepared from a common sample of raw milk.

Date	Pre-sterilization heat treatment of milk				Total solids	Heat stability	Viscosity at 30° C. (86° F.) after storage at 30° C.				
	Raw milk		Concentrated milk				No storage	10 days	30 days	225 days	410 days
	Temp.	Time	Temp.	Time							
12/2/41	°C.	min.	°C.	min.	%	min.	c.p.	c.p.	c.p.	c.p.	c.p.
12/29/41	95	10	26.0	44	26	12	13	13
2/13/42	140	1	31.7	23	174	95	47	51
	95	10	26.0	43	30	19	16	24
6/25/42	120	2	34.9	25	460	222	156	360
	95	10	27.7	23	58	24	19	18	15
	95	10	120	0	27.7	34	25	16	15	14	15
	95	10	130	0	32.5	44	66	43	37	53	55
7/9/42	95	10	140	0	37.2	35	165	132	121	213	191
	95	10	120	4	35.0	22	207	165	170	197	290
7/13/42	95	10	27.6	29	45	18	16	15	17
	95	10	120	0	27.6	46	24	14	13	18	20
	120	1/4	27.6	36	34	14	13	16	49

milk was greatly influenced by the heat stability of the sample while one of the important factors affecting stability was concentration. The effect of heat stability upon the viscosity of some milks of different concentrations is shown also in table 1.

Results which show the extent of the viscosity changes in evaporated milk subjected to different manufacturing and storage conditions are presented in figures 4 and 5 and in table 1. The important changes during storage

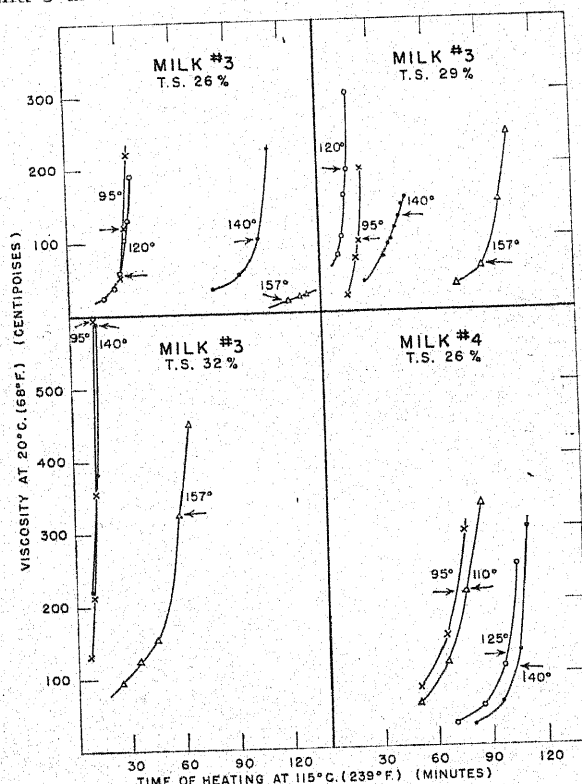


FIG. 2. The relationship between forewarming temperature, heat stability and the development of viscosity in two evaporated milks during sterilization. The solids content of the milk is indicated in each quadrant. The forewarming temperature is given for each curve; the forewarming time of the 95° C. samples was 10 minutes while for the other samples it was 25 seconds. The arrows indicate the time of the appearance of visible coagulation.

were: a thinning of all samples during early storage, then a period of only slight viscosity change, and finally a thickening in some of the milks.

The initial loss in body in all the milks tested was retarded by low storage temperatures. Samples of a representative 26 per cent solids milk held 55 days at 25° C. decreased in viscosity to the same extent as other samples held 8 days at 40° C. Samples held 55 days at 16° C. had the same viscosity as those held 5 days at 25° C.

Data on the effect of aging whole milk upon the viscosity of its 26 per cent evaporated milk were obtained. Fresh milk was held at 37° C. for 2 hours during which time the acidity increased from 0.18 to 0.21 per cent. Aging the fresh milk affected the viscosity of its evaporated product indirectly through its effect upon heat stability. Thinning of the milks during storage did not appear to be caused by the aging treatment.

The differences in the kind of body which may be expected in storage samples of evaporated milk of approximately 30 per cent solids content is

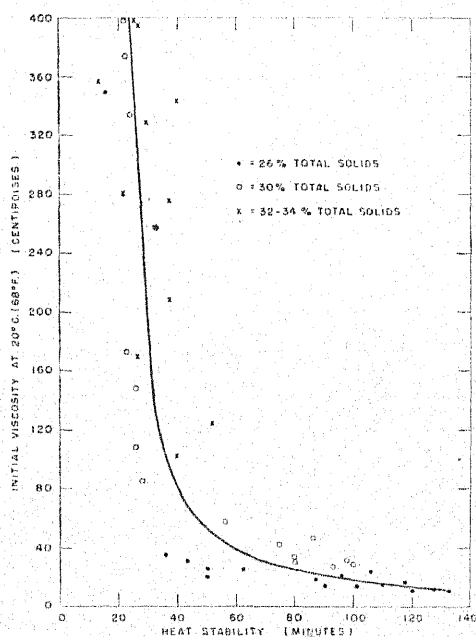


FIG. 3. Viscosities of evaporated milks of different heat stabilities measured after completion of the normal sterilizing process. Each milk was cooked for 18 minutes at 115° C. (239° F.), cooled to 20° C. and the viscosity determined within 3 hours after processing.

shown in figure 4. Milk A showed progressive thickening after completion of the initial thinning reaction, while milk B remained substantially unchanged in viscosity during the storage period.

Data on forewarming treatment, solids content, heat stability and viscosity of six batches of milk are given in table 1. The samples were stored and viscosity measurements were made at 30° C. The loss in the viscosity of these samples after 10 days' storage at 30° C. averaged 41 per cent of the original viscosity values. There was an additional average loss of 10 per cent of the original values during storage between the 10-day and 30-day periods. These losses are of the same order as those reported by Mojonnier and Troy (4).

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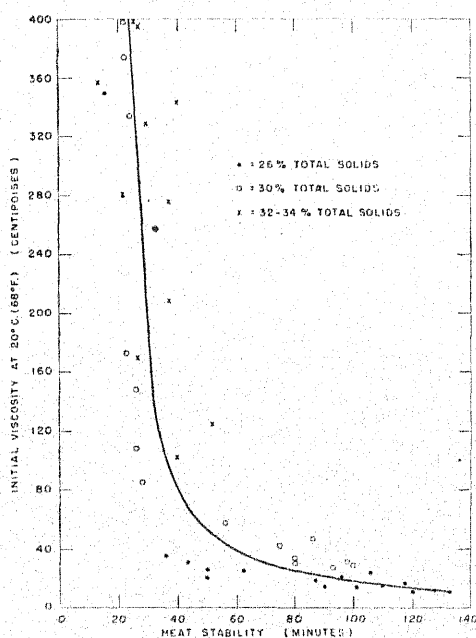


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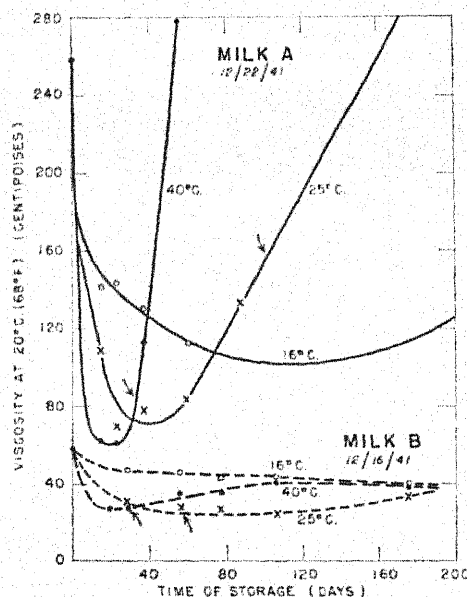


FIG. 4. The effect of time and temperature of storage upon the viscosity of two evaporated milks. The time of observance of heavy fat separation is indicated by arrows. Only moderate fat separation was noted in the 16° C. samples at the end of the storage period. Sample 12/16/41 was forewarned at 140° C. (284° F.) for 30 seconds, contained 30.0 per cent solids and had a heat stability of 56 minutes. Sample 12/22/41 was forewarned at 130° C. (266° F.) for 30 seconds, contained 31.7 per cent solids and had a heat stability of 33 minutes.

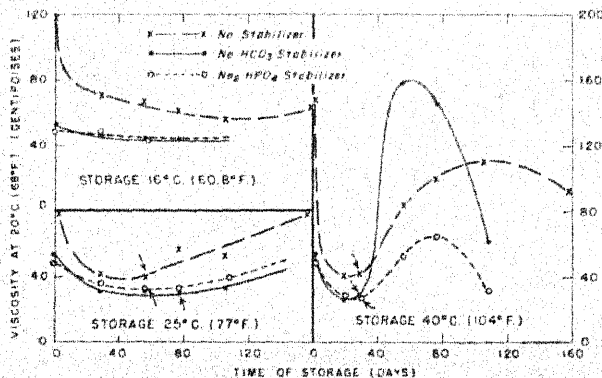


FIG. 5. The effect of time and temperature of storage upon the viscosity of samples of an evaporated milk sterilized with and without stabilizers. The time of observance of heavy fat separation is indicated by arrows. Only moderate fat separation was noted in the 16° C. samples at the end of the storage period. The milk was forewarned at 95° C. for 10 minutes before concentration to 30 per cent solids content. The heat stabilities of the samples were: no stabilizer—26 min., bicarbonate stabilizer (4½ oz. per 1000 lbs. milk)—34 min., phosphate stabilizer (18 oz. per 1000 lbs. milk)—37 min.

The effect of large quantities of two stabilizing salts on the body changes of an evaporated milk during storage at three temperatures is shown in figure 5. This milk was representative of six samples upon which data of this nature were obtained. The high viscosity of the control sample can be attributed to its low stability.

Observations on fat-separation during storage were made on the evaporated milk samples in conjunction with the viscosity determinations. Figure 6 shows in a general way the relationships between temperature and time of storage. The curve for excessive fat-separation was based on visual observations; exact measurements were not made. If it is considered that cans of evaporated milk should be turned when they have stood half the time

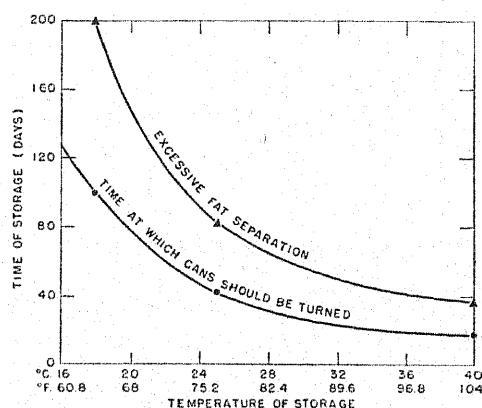


FIG. 6. The effect of temperature and time of storage upon fat separation in evaporated milks. The upper curve is based on average values from 20 milks of 26-32 per cent solids content held in undisturbed storage at different temperatures. The lower curve is an estimate of the time at which cases of evaporated milk in storage should be turned.

necessary to cause excessive fat-separation, the lower curve of figure 6 may be drawn. It would, therefore, seem reasonable to require the turning of cases of evaporated milk at some regular interval which lies below the lower curve of figure 6. For example, at 25° C. (77° F.) the milk should be turned every 6 weeks.

DISCUSSION

The viscosity of evaporated milk passes through well-defined changes arising from the manufacturing processes and the storage conditions to which it is subjected. There is first a thickening during the heat of sterilization, then a thinning during early storage and finally, after long periods of storage, the viscosity may again increase until a gel has formed.

The rate of thickening of evaporated milk during the sterilization process is variable, being the greatest shortly after heating is begun and immediately before coagulation appears. While the rapid thickening immediately pre-

ceeding visible coagulation is, no doubt, a part of the coagulation process, all milks do not attain the same body before coagulation begins. The attainable body at coagulation seems to depend primarily upon the heat stability of the milk. Heat stability, in turn, is largely influenced by the concentration of milk solids, the conditions of forewarming, and the ionic equilibrium which exists in the serum.

The results of a number of experiments with milks differing in quality and forewarmed at different temperatures indicate that the relationship between the attainable viscosity at coagulation and the heat stability of the evaporated product is of a reciprocal nature.

Thickening in evaporated milks during sterilization does not proceed rapidly until about 10 minutes before coagulation. A processing period of about 20 minutes is generally favored in commercial practice. If a heavy, creamy body is to be developed, the heat stability of the milk should not exceed 30 to 40 minutes. When the cooking time to be used approaches the heat-stability time, extensive increases in viscosity may be encountered. Figures 1 and 2 show the thickening for which each minute of heating may be responsible when the milk is in the pre-coagulation phase. Milks with stabilities in excess of 50 minutes will be exceedingly thin after heating them at 115° C. for 20 minutes unless an increase in solids content is depended upon to build body.

The first change which occurs in the body of evaporated milk during storage is a rapid thinning, an effect which is accelerated at high storage temperatures. As thinning progresses a viscosity level is finally reached which may be termed the basic storage viscosity. In this region the viscosity ceases to decrease so rapidly; there is a distinct flattening in the viscosity curve (figs. 4 and 5, and table 1). As the milk loses body, another storage change, a separation of the fat, begins.

During storage, control of the body of evaporated milk should be first concerned with methods for delaying the initial thinning process and establishing a relatively high basic storage viscosity. Attainment of the basic storage viscosity may be effectively delayed by subjecting the milk to low storage temperatures. When evaporated milks were stored 60 days at 20° C. (68° F.) or lower, the loss in viscosity was less than the loss which occurred after 10 days at 40° C. (104° F.). Evaporated milks subjected to storage temperatures above 30° C. (86° F.) often reached their basic storage viscosity in 10 days.

Although the level of the basic storage viscosity was determined largely by heat stability, it was also influenced by the solids content of the milk. The 37 per cent solids milk of June 25, table 1, had a stability of 35 minutes and a viscosity after 30 days' storage of 121 centipoises. A portion of the same milk forewarmed at 120° C. (with no holding time) to give the same stability (34 minutes) at 27 per cent solids content had a viscosity of only 15 centipoises after 30 days' storage.

No manufacturing treatment, such as alteration in the type or conditions of forewarming, has yet been found which proved capable of preventing the initial rapid loss in the viscosity of evaporated milk during storage at high temperatures.

The last stage in body deterioration of evaporated milk consists of a thickening of the milk, which sometimes reaches the point of gelation. Storage thickening is rarely found in the commercial product, partly because much of the milk is consumed before thickening sets in and partly because some milks apparently never would reach this phase of development. Before thickening becomes objectionable an excessive fat-separation can generally be observed. The viscosity increase during storage usually starts too late to be very helpful in retarding fat-separation.

Certain samples, such as milk B, figure 4, did not thicken. The storage viscosity curves of many 26 per cent evaporated milks followed the course of milk B. High-solids milks thickened more readily than did 26 per cent milks but the greatest increase occurred in the solids range above 32 per cent.

The use of sodium bicarbonate as a heat stabilizer (fig. 5) accelerated storage thickening in several milks but not before a heavy layer of fat separated from the samples. Sodium bicarbonate is no longer used as a stabilizer in the manufacture of evaporated milk.

Evaporated milks which have been subjected to sterilization processes requiring only a minute or two to complete and consequently which have little cooked flavor and color are known to thicken quickly during storage. Evidently the drastic heat treatment of the commercial product retards storage thickening while the mild treatment given various experimental batches permits it.

Little is known of the real cause of thickening in evaporated milk during storage. It may be a slow continuation of the coagulation process accompanied by an orientation of the caseinate molecules which finally produces an irreversible gel structure. Molecular orientation is suspected as being involved because undisturbed storage appears to promote thickening and the early incipient gel structure may be easily reduced by shaking.

It is hoped that future investigations on the viscosity of evaporated milk will eventually indicate a means of controlling the thinning and thickening processes so that a good body in evaporated milk can be produced and permanently held during storage.

SUMMARY

1. The viscosities of evaporated milks of 26 to 36 per cent solids concentrations followed a well-defined pattern during processing and storage. Thickening occurred during the sterilization process; this was followed by a loss of body and a thinning early in the storage period. The low storage viscosity was maintained for various and unpredictable lengths of time, dur-

ing which fat-separation occurred. Late in the storage period a final thickening, which often proceeded to gelation, sometimes set in.

2. The rate of thickening of evaporated milk during sterilization at 115° C. (239° F.), although variable, becomes greatest in the 10-minute period immediately preceding coagulation. A heavy, creamy body could be developed during this pre-coagulation stage. In general, the lower the heat stability of a milk, the higher the viscosity it would develop during the 10-minute period preceding coagulation. Concentrated milks of high heat stability which reached the end of the sterilization period before entering the thickening phase did not develop, during processing, the high viscosity shown by milks of lower heat stability.

3. The body formed during the sterilization of milks of different concentrations became greater as the solids content of the milk was increased. The heaviest viscosities were produced in milks of high concentration and low heat stability.

4. Variations in milk quality and in forewarming procedures affected the viscosity of evaporated milk indirectly through their effect upon the heat stability of the milk.

5. The magnitude of the decrease in the viscosity of evaporated milk during early storage was dependent upon the storage temperature. At temperatures below 16° C. (60.8° F.) the loss of body was small. At temperatures above 30° C. (86° F.) evaporated milk sometimes lost 40 per cent of its original viscosity during the first 10 days of storage.

6. After the initial thinning in the viscosity of evaporated milk held in storage at constant temperature, a basic storage viscosity level was reached beyond which the rate of viscosity loss during prolonged storage was small.

7. Some evaporated milks, especially those receiving light heat treatments and those with a high concentration of solids, began to show increases in viscosity even to the point of gelation, late in the storage period.

8. The results indicate that the procedure for developing and maintaining a satisfactory body in evaporated milk which is used by many manufacturers conforms to the best practice that can be devised from our present state of knowledge. Assuming efficient homogenization and uniform handling of the canned product, important steps in body control are: (a) adjust the heat stability to a value of a few minutes greater but not more than twice the cooking time, bearing in mind that viscosity increases rapidly during the 10 minutes preceding coagulation; (b) store the finished milk at a temperature below 21° C. (70° F.); (c) turn the cases approximately every six weeks; (d) the viscosity may be increased by raising the milk solids content of the product.

ACKNOWLEDGMENT

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THE BACTERIOLOGY OF BULL SEMEN. II. THE EFFECT OF BACTERIA UPON RAPID TESTS FOR SEMEN QUALITY

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In a recent report Beck and Salisbury (1) have proposed two rapid methods for estimating the quality of bull semen; namely, the rate of methylene blue reduction by semen in yolk-citrate diluent and the survival of spermatozoan motility during short-time-high-temperature incubation. The following work was undertaken to determine the effect of bacterial contamination upon the accuracy and validity of these quick tests, especially the specific effect on the methylene blue reduction rate. The effect upon the methylene blue test was studied by two methods: first, by calculation of the multiple correlation coefficients to make allowance for the reduction due to spermatozoa and other known factors (1) in semen samples; and, second, by determining the methylene blue reduction time when pure cultures of bacteria were inoculated into yolk-citrate diluent, with and without seminal plasma. The effect of the short-time-high-temperature incubation test upon the bacterial content of semen has also been determined.

A preliminary estimate of the effect of bacteria upon the methylene blue reduction test may be obtained by an examination of the data available for other biological fluids, as for example milk. Thornton and Hastings (7) using 1:200,000 methylene blue at 37° C. found the bacterial count at the time of complete reduction to range from 3.5 to 45 million bacteria per ml. No sample with an initial count of fewer than 20 million bacteria per ml. gave a reduction time of less than one-half hour. In this and similar work the factors shown to affect the reduction time are the type of bacteria, their physiological condition, the temperature, the nature of the substrate present, and the concentration of methylene blue to be reduced. That storage may also be a factor was shown by Fayer (3) who stored milk samples at 4° C. for 18-72 hours and found a decrease in the rate of methylene blue reduction without a change in bacterial count. Bacterial counts of the magnitude usually found in semen samples would not on the basis of the findings of Thornton and Hastings (7) be expected to affect the proposed methylene blue reduction test.

EXPERIMENTAL

Methods

The semen samples used in these experiments were obtained under routine conditions either from bulls in the dairy herd at Cornell University

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or from bulls owned by the New York Artificial Breeders' Cooperative in Syracuse, New York. All semen samples were collected with the artificial vagina. The semen was handled during examination, dilution and preparation for storage after the methods described by Willett and Salisbury (8). The methylene blue reduction tests were carried out as previously described (1). Briefly the test involves an observation of the time required for the complete reduction of 1:40,000 methylene blue by bull semen diluted at a constant rate with yolk-citrate diluent. With the exception of the experiments to determine the effect of various temperatures on the reduction time and upon bacterial numbers, the tests were run at 45° C. Bacterial counts were made on 2 per cent blood agar plates incubated 3 days at 37° C.

The effect of bacteria present in semen on the reduction test

For determination of the effect of bacteria, freshly diluted semen samples were selected from the bulls found in earlier studies (4) to yield semen containing the highest bacterial counts. Semen from these bulls was also of poorer than average quality in other respects; *i.e.*, long methylene blue reduction times had been noted. These samples were selected as those most likely to give erroneous estimates of semen quality if bacteria have a significant effect upon the methylene blue reduction test.

A factorial experiment was designed to simultaneously study the effect of the number of bacteria on the methylene blue reduction time at several temperatures and the effect of a 45-minute incubation period at these temperatures on the bacterial count. Samples of the same ejaculates were stored for 2, 4, 6, 8, and 10 days, following which bacterial counts and methylene blue reduction tests were run. Aliquots of the same samples were incubated and stored with and without methylene blue. In this way the effect of high-temperature incubation, apart from methylene blue, upon the bacterial count was determined. The methylene blue was added to one series of stored samples with the thought that if the reduction time was not influenced by bacterial growth, or other factors, the relative metabolic activity of the semen could be determined at any time during storage by simply warming a sample to the proper temperature and observing the reduction time.

For each ejaculate the spermatozoan concentration was determined at the time of collection and the motility was observed immediately before each methylene blue reduction test was performed. Thus, it was possible to determine the multiple effects of storage interval, spermatozoa count, motility, and bacterial count on the time required to reduce methylene blue. Ten ejaculates were used and 21 individual plate counts, motility estimates and methylene blue reduction tests were performed on each diluted ejaculate. It was later found necessary in the statistical analysis of the storage experiment to remove the data for two ejaculates as the semen was of such poor quality that it did not reduce the methylene blue in the time allotted for the

incubation period. The removal of these data had no influence on the conclusions drawn from these experiments.

For fresh samples the correlation coefficients between methylene reduction time for each of the following was: spermatozoan concentration, -0.7780 ; spermatozoan motility, 0.3431 ; and bacterial count, -0.1427 . The coefficient for spermatozoan concentration is the only significant observation ($n = 10$). Though these fresh samples contained a wide range of bacterial counts the number present have but a minor influence on the methylene blue reduction time. The stored samples showed even wider variation in bacterial count. With these samples the calculated correlation coefficients for each of the following was: spermatozoan concentration, -0.6126 ; spermatozoan motility, -0.3110 ; and bacterial count, -0.1022 ($n = 48$). The first two coefficients are statistically significant, the last is not. Even with the larger number of observations the data indicate that the part played by bacteria in the reduction time is not large. All samples contained predominantly diphtheroid organisms, except one ejaculate in which the majority of the organisms were *Pseudomonas aeruginosa* (*pyocyaneus*).

The mean data for the fresh and stored samples are shown in tables 1 and 2. The methylene blue reduction times for fresh semen are too short for bacterial multiplication to be an important factor, when the tests are run at temperatures above 45°C . as recommended. In fact, the experiments show (table 1) a decrease in bacterial count at temperatures of 45°C . and above. The decrease is especially marked in the presence of methylene blue.

The stored samples (table 2) showed, on the average, a slight decrease in bacterial count over the first 4 days followed by an increase. Over this period, the methylene blue reduction time increased slightly and then decreased. The correlated changes are, however, too slight to be of significance.

The effect of methylene blue on the numbers of bacteria

From table 1 a comparison of the numbers of bacteria found in semen samples after 45 minutes incubation with and without methylene blue may be obtained. The samples used had stood at the laboratory temperature for about 30 minutes before incubation, which time was found to be the average required to set up the experiments. In table 2 may be seen a similar comparison of bacterial counts made on the same ejaculates after storage at 5°C . The methylene blue had been added to one series of samples before storage, and to the other just prior to the 45-minute incubation period. As limited reduction had proceeded in the samples to which methylene blue was added before storage, the reduction times were shorter than for the samples to which the reagent was added just before incubation.

It will be noted that incubation of the fresh semen in the presence of methylene blue reduced the bacterial count in all cases. In the absence of methylene blue, the count decreased only at the two higher temperatures. An analysis of variance of the data showed these differences to be highly

TABLE 1
The effect of incubation temperatures on the bacterial count of semen samples

No. samples	Sper- matozoa	Motility fresh	Bacterial count	Mean methylene blue reduction time during and mean bacterial counts after 45 minutes' incubation at											
				37.5°		40.0°		42.5°		45.0°		47.5°			
				Time	Count	Time	Count	Time	Count	Time	Count	Time	Count		
8	thousands per mm. ³	%	thousands per ml.	min.	thousands per ml.	min.	thousands per ml.	min.	thousands per ml.	min.	thousands per ml.	min.	thousands per ml.		
	1109	66.2	640	31.5	553	29.6	624	12.1	561	9.7	463	9.9	316		
Mean bacterial counts after 45 minutes' incubation without methylene blue present															
					879		897		881		599		503		

TABLE 2
Mean reduction time and bacterial count after storage at 5° C.

Storage period days	Spermatozoa motility %	Methylene blue reduction time min.	Bacterial count after 45 minutes at 45° C.	
			Without methylene blue thousands per ml.	Methylene blue added before storage thousands per ml.
0	66.2	9.7	640	640
2	47.5	14.7	430	363
4	37.5	12.9	397	237
6	31.2	11.1	1,141	567
8	30.0	12.1	3,171	1,054
10	25.0	13.2	2,888	2,279

significant. These facts support the use of 46.5° C., previously suggested by Beck and Salisbury (1), for the short-time-high-temperature incubation and methylene blue reduction tests. These workers have shown a very high correlation between the decrease in motility at 46.5° C. in 1 hour and the decrease in motility during 10 days' storage at 5° C. The bacterial count for samples stored with methylene blue was slightly lower in all cases than samples stored without methylene blue, but the difference was so small as to suggest that this was only a chance deviation. In the previous report (1) it was shown that the amount of methylene blue used did not have a detri-

TABLE 3

Methylene blue reduction test on pure cultures of bacteria in yolk-citrate diluent

Organism added	Days stored at 5° C.					
	0		5		10	
	Bac- terial count	Reduc- tion time	Bac- terial count	Reduc- tion time	Bac- terial count	Reduc- tion time
	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>
<i>Escherichia coli</i>	130.0	0.6	11.0	100.0
	1,500.0	31	15.0	40	13.0	> 120.0
<i>Aerobacter aerogenes</i>	900.0	> 120	48.0	320.0	7.5
			750.0	11	1,000.0	4.5
<i>Pseudomonas aeruginosa</i>	800.0	> 120	750.0	36.0	10.0
			9.0		120.0	6.0
<i>Staphylococcus aureus</i>	1,200.0	> 120	0.18	9.0	14.0
			15.0	6.0
Diphtheroid strain 1	2.0	0.13	0.3
2	7.0	21.0	4.0
	<i>thou- sands per ml.</i>	<i>min.</i>	<i>thou- sands per ml.</i>	<i>min.</i>	<i>thou- sands per ml.</i>	<i>min.</i>
None (control)	0.03	0.3	4.0

mental effect on the maintenance of spermatozoan motility in stored samples. From these observations it has been suggested that this dye could be used to identify the semen of one breed of bulls from that of another and thus avoid field errors which sometimes arise in artificial insemination.

The effect of specific types of bacteria on methylene blue reduction in yolk-citrate diluent

To test directly the effect of bacteria, yolk-citrate diluent and yolk-citrate diluent plus seminal plasma were inoculated with pure cultures in numbers higher than those usually found in semen and the methylene blue reduction test performed at 45° C. These tests were, as previously mentioned, performed within 30 minutes of inoculation. Other inoculated samples were stored for 5 and 10 days before reduction tests were performed. Twenty-

hour cultures of organisms isolated from semen samples were used as inoculum. These organisms were encountered in large numbers in various samples of semen during the course of the study. The data in tables 3 and 4 indicate that in fresh samples, none of the organisms would interfere with the test even when present in numbers as high as 1 billion per ml. The shortest reduction time (30 minutes) which was obtained with 1.5 billion *E. coli* was still beyond the reduction time for all but the poorest semen samples.

The results with samples stored for several days were not as satisfactory. If bacteria, which grow at the storage temperature, are present the reduction

TABLE 4
Methylene blue reduction test on pure cultures of bacteria in yolk-citrate plus seminal plasma

Organism added	Days stored at 5° C.					
	0		5		10	
	Bacterial count	Reduction time	Bacterial count	Reduction time	Bacterial count	Reduction time
	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>	<i>millions per ml.</i>	<i>min.</i>
<i>Escherichia coli</i>	48.0	60	54.0	48	75.0	9.0
	6.0	7.0
<i>Aerobacter aerogenes</i>	17.0	120	990.0	12	2,000.0	6.0
<i>Pseudomonas aeruginosa</i>	60.0	120	66.0	120	60.0	6.5
<i>Staphylococcus aureus</i>	8.0	120	30.0	120	20.0	16.0
Diphtheroid strain 1	3.0	6.0	4.0	15.0
2	0.02	0.03	0.05	40.0
	<i>thousands per ml.</i>	<i>min.</i>	<i>thousands per ml.</i>	<i>min.</i>	<i>thousands per ml.</i>	<i>min.</i>
None (control)	0.015	0.009

time may be very short. Cultures of *Aerobacter aerogenes* and *Pseudomonas aeruginosa* not only grow at 5° C. but the citrate present in the diluent is an excellent substrate for these organisms. When 750 million or more *Aerobacter aerogenes* organisms per ml. are present (tables 3 and 4), they would interfere markedly with the validity of the methylene reduction test after 5 days' storage at 5° C. After 10 days' storage counts of a hundred million of either of these two species of bacteria reduced the methylene blue fast enough to interfere with the test. When the seminal plasma was added to the yolk-citrate short reduction times were observed with each organism after a storage period of 10 days. The effects of the bacterial types contributed by the seminal plasma were not determined. However, as one may observe from the tables the number of bacteria in the plasma was very low. The range of bacterial numbers, in fresh diluted semen, the mean of which is shown in table 1, was from 97 thousand to 4.5 million.

After 10 days' storage the average count had increased to about 3 million bacteria per ml. with one count as high as 60 million. In only three diluted semen samples were the numbers as high after 10 days' storage as was found in the inoculated material. In three ejaculates the count increased as much as 20-fold, while in three others the total count decreased during storage. Greater increases in count were reported in a recent publication of the United States Department of Agriculture (5). With higher initial counts these workers report a 10-fold increase in bacterial count during 7 days and a 65-fold increase during 10 days' storage at 4° C.

A study of the data of this experiment reveals that only part of the short reduction times observed for stored yolk-citrate may be attributed to bacterial numbers. For diluent, or diluent plus seminal plasma, stored 10 days, short reduction times often were found where the numbers of bacteria present was no higher than those which in fresh yolk-citrate required more than 2 hours to reduce the dye. Apparently some factor other than numbers of bacteria present is partially responsible for the short reduction times observed for stored, inoculated material.

In this connection it should be pointed out that the negative correlation between spermatozoa count and methylene blue reduction time continued at the high level previously reported (1) regardless of the length of storage interval. Such definite trends were not noted for the relation between motility of the spermatozoa and methylene blue reduction time as the storage interval advanced. For the present, the methylene blue test for quality of semen should be used only with fresh semen and fresh diluent for the most satisfactory results.

DISCUSSION

From the results of these investigations it is concluded that if bull semen is collected and handled with proper sanitary precautions, and if the diluent is prepared following a definite aseptic routine (4) the number of bacteria in fresh semen and diluent will not interfere with the estimates of semen quality obtained with the methylene blue reduction test. In addition, the temperature recommended for conducting the test, 46.5° C., is high enough that bacteria are unable to grow. In fact, such a temperature has a lethal effect on the bacteria, especially in the presence of methylene blue.

Of the bacteria most likely to interfere with the test, the coli-aerogenes organisms can be largely excluded by sanitary care in preparation of equipment and during collection and handling of samples. The exclusion or control of the number of pseudomonas organisms is more difficult. These bacteria are sometimes found in large numbers and as the predominating type in the semen of some bulls. Ordinary methods of control do not influence the numbers of these bacteria for their source appears to be deep in the reproductive tract. Bulls harboring *Pseudomonas aeruginosa* in their reproductive tracts are likely to have poor breeding records (2, 4) and are poor risks for artificial insemination.

For stored samples of diluted semen the methylene blue reduction test should be used with caution. Certain unknown factors, in addition to the apparently minor effects of the number and kinds of bacteria, influence the test with semen samples stored more than 2 days so that reliable conclusions may not be drawn. For estimation of the quality of samples stored for 4 or more days observation of the duration of motility at 46.5° C. should give reliable estimates of potential livability of spermatozoa during continued storage at 5° C. Further work needs to be done before the factors influencing the reduction test in samples of diluted semen stored more than 2 to 4 days can be established.

SUMMARY

1. When sanitary precautions are observed, the number of bacteria found in freshly drawn semen or freshly prepared yolk-citrate diluent is not sufficient to interfere with the methylene blue reduction test for semen quality.
2. The short-time-high-temperature incubation test for semen quality may kill up to 50 per cent of the bacteria present when the test is run at 45° C. or above in the presence of methylene blue. A temperature of 46.5° C. is recommended.
3. The methylene blue reduction test is not recommended as a criterion of the quality of semen stored more than 2 days.
4. The short-time-high-temperature incubation test is recommended as a criterion of continued livability of the spermatozoa in stored semen samples.
5. Though the bacterial population of stored samples can be controlled by proper precautions, factors other than the number of bacteria, and the activity and concentration of the spermatozoa are involved in the methylene blue reduction rate of stored samples.

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PEPPERGRASS SILAGE

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Recently attention has been directed toward the use of weed crops for forage purposes. At the Virginia Station alfalfa and brome grass were established to serve as supplementary pasture crops to be available in July and August when bluegrass is in low production. The alfalfa had been pastured off by May 20th and there was a heavy stand of peppergrass (*Lepidium virginicum*) remaining ungrazed. There was entirely too much of this weed to permit it to go to seed. If clipped and allowed to remain on the ground the peppergrass would smother out the alfalfa. Mowing, raking and hauling away the peppergrass would be costly if no use were made of it.

Ten acres of peppergrass, yielding about 28 tons, were cut and ensiled with 50 pounds of molasses per ton as a preservative after due consideration of the possibility that the silage might be strong with peppergrass flavor.¹ Five acres were cut from the alfalfa field and some alfalfa was picked up by the mowing machine. The other five acres were cut from the brome grass field and some brome grass was clipped. It was estimated that the ensiled material was 80 per cent peppergrass. The seeds were immature but had the field been mowed and the peppergrass left on the ground the seeds would probably have matured sufficiently to germinate. When the material was cut into the silo there was sufficient volatile material in the air to irritate the eyes. The ensiled material kept well. After the silage had undergone fermentation there was no trace of the peppergrass flavor or odor remaining. Cows ate up to 50 pounds per day of the silage and appeared to relish it better than silage made from bluegrass with corn meal as a preservative. Samples of peppergrass in as nearly as possible the same stage of maturity as that which was ensiled were submitted to the Agricultural Chemistry Department for analysis. A sample of peppergrass silage containing about 20 per cent alfalfa was taken from the silo and submitted also. The analyses of these materials are shown in table 1 with the analysis of corn silage for comparison. Table 2 shows that the quality of peppergrass silage was satisfactory as there was no decline in production due to peppergrass silage. Cow number 6 was nearly dry and therefore any change in the feed would not have prevented a decline in the milk production of the cow.

The milk had no taste of peppergrass or indole. When the milk was

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¹ Hussong, R. V., and Quam, Sidney. Relationship of Consumption of Peppergrass by Cows to the Flavor and Indol Content of Butter. *JOUR. DAIRY SCI.*, 26, No. 6: 505-513. 1943.

TABLE 1

Analyses of peppergrass and peppergrass silage compared with corn silage

	Corn silage* (dent, well matured, well cared)	Peppergrass	Peppergrass silage
	%	%	%
Dry basis			
Protein	8.56	12.86	16.19
Fat	3.08	5.54	4.40
Crude fiber	21.92	34.17	23.63
Ash	5.48	9.32
Nitrogen-free extract	60.95	38.11	40.62
Total dry matter	29.29	36.47	28.75
Phosphorus	0.21	0.23	0.42
Calcium	2.74	1.34	1.21
Potassium	0.96	3.00	2.41
Magnesium	0.40
Silica	1.28

The peppergrass was analyzed by Mr. H. H. Hill and the peppergrass silage by James F. Eheart, both of the Agricultural Chemistry Department. The peppergrass contained approximately 20 per cent alfalfa.

* The values on the dry basis are computed from data of Morrison's Feeds and Feeding, 20th Edition.

TABLE 2

The milk production of cows fed bluegrass silage and peppergrass silage

Cow No.	Bluegrass silage		Peppergrass silage	
	Amounts of milk produced			
	May 2-16	May 17-31	June 1-15	June 16-30
	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1	393.0	350.0	313.5	332.5
5	476.5	429.0	431.5	455.0
6	274.0	183.0	143.5	108.0
7	400.0	346.5	321.0	336.5
Average daily milk flow for 4 cows	27.6	23.4	21.6	22.0

The rate of decline of milk flow was reduced when bluegrass silage was replaced with peppergrass silage.

TABLE 3

The quality of butter made from milk produced by cows fed peppergrass silage

Lot No.	Age of cream in days	Condition of cream at churning	Butter
1	7	High acidity	Salted, old cream flavor, no peppergrass flavor
2	7	Low acidity	Unsalted, superior flavor, no peppergrass flavor
3	1	Sweet	Unsalted, excellent flavor, 92 score

separated the cream had a normal flavor. Butter was made from the cream without using a starter with the results shown in table 3.

Three judges scored the butter samples and no off-flavors attributable to the peppergrass were detected.

Evidently the substances producing the peppery flavor of the green material were oxidized in the fermentation process. The growing of peppergrass for silage is not advocated; however, crops that contain peppergrass may be ensiled without fear that dairy products will have an off-flavor as a result of their use.

One hundred peppergrass seeds were taken from the silage two weeks after ensiling for a germination test. There was no germination. Furthermore there was no germination of seed taken from the silo in early September, after the elapse of a normal rest period for these seeds.

SUMMARY

Ensiling peppergrass (*Lepidium virginicum*) with molasses as a preservative produced a palatable silage which after fermentation had no peppergrass flavor. Neither cream nor butter produced from the milk of cows fed this silage showed any off-flavor. Peppergrass seeds did not germinate two weeks after ensiling. Crops containing peppergrass may be ensiled without fear that off-flavor will result in the milk or milk products.

ACKNOWLEDGMENT

The assistance of Dr. C. C. Flora in producing the butter and judging the butter samples is gratefully acknowledged.



A NEW QUANTITATIVE METHOD FOR ESTIMATION OF TOTAL COMBINED LENGTH OF MOLD FRAGMENTS IN BUTTER¹

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The method described in this report resulted from studies on retention of mold fragments (mycelia)² by butter, buttermilk and wash water during manufacture of butter.

During these and previous studies (3) it became apparent that a more quantitative method for estimating content of mold hyphae or filaments in butter and other products would have to be developed before information could be obtained on mold retention by the various materials studied. Since the "mold mycelia value" of butter by the official method now in use is influenced primarily by the total mass of mold growth present, the logical quantitative method would be one that would measure the total length of all combined mold fragments in a measured sample. The difficulty of extracting the mold fragments renders a macroscopic approach impossible. The possibility of analyzing for some chemical constituent contained by the mold hyphae and foreign to butter appears remote at the present time. About the only possibility remaining is a microscopic method. The Wildman method (4) in use at present provides a comparative rather than quantitative value because it does not measure the total length of the combined filaments in a sample. A method was therefore developed in which the total combined length of the mold hyphae visible with low power (100×) magnification of the microscope was determined and expressed in millimeters per gram or mgm. Because the method has proven so convenient for certain studies on mold content of butter and other materials, it is being published with the hope that it may prove useful to other investigators in this field.

DESCRIPTION OF METHOD

1. Thoroughly mix 1 g. butter with 9 ml. hot (70-75° C.) gum solution. (It is desirable to scrape off about one-eighth inch of the surface butter and

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² The term "mold mycelia" now commonly used in referring to the amount of dead mold filament in butter should perhaps, technically speaking, be changed to "mold hyphae" or "fragments of mold hyphae." A mold hypha (plural—hyphae) is a single thread or filament of mold. A mold mycelium (plural—mycelia) consists of a mass or network of interlacing filaments or hyphae. Therefore it is the filaments or hyphae or hyphae fragments that are counted in the Wildman method. Filaments, hyphae and mold fragments are used synonymously in this paper.

then obtain the one gram sample by scraping it from an area of a few square inches.)

2. Transfer 0.1 ml. to one side of a 1×3 inch (26×77 mm.) slide.

3. Spread over exactly one-half of the slide. (One means of evenly distributing the preparation is to spread the material with a small, solid glass rod, first lengthwise and then crosswise. The preparation seems to distribute itself more evenly if, after spreading on the slide, it is placed for a few minutes on a level surface at room temperature before setting on a warm surface to dry.)

4. Dry on a warm surface.

5. Stain with Newman's (1) or a Modified Newman's stain. (The same precautions regarding drying of stains before washing to remove excess dye, should be observed as in the staining of milk films with Newman's stain. It is possible to wash the slides too long in removing the stain. A few trials will indicate the amount required. Nevertheless some mold hyphae fragments will not stain intensely and a knowledge of the microscopic appearance of mold hyphae is helpful in identifying them.)

6. Calibrate factor for low power objective of microscope. Include dilution of sample and area of slide.

7. Examine 25 or preferably 50 fields on each slide. If the sample contains much mold, 25 fields may be sufficient. Estimate, by means of a ruled ocular micrometer disc, the total length of mold filament for each field. (Appearance of the preparation under the microscope is often improved by covering it with a thin film of immersion oil. This is particularly true where the method has been applied to substances containing considerable protein, such as cream or buttermilk.)

8. Calculate average length of filament per field.

9. Multiply average length of filament per field by the factor.

10. Express results as millimeters per gram or preferably millimeters per mgm.

SAMPLE CALCULATION

A. Diameter of field = 1.66 mm.

B. Area of field = $\pi r^2 = 2.164$ sq. mm.

C. Area of one-half slide = 1001 sq. mm.

Number of fields in one-half slide = 463.

D. Butter diluted 1:10; 0.1 ml. placed on slide.

Total dilution = 100 times.

E. Factor = $C \times D = 46,300$.

F. Average total mm. mold filament per field of sample preparation = 0.346.

G. Mm. mold per gm. = $E \times F = 16,020$.

H. Mm. mold per mgm. = $\frac{E \times F}{1000} = 16.02$.

Some comments might be made on certain steps in the procedure. The diluting medium was a 0.75 per cent carob bean gum solution similar to that used in the Wildman method (4). Other gum solutions that provide sufficient viscosity and a reasonably clear solution should be satisfactory. Among the preparations also used in these studies were the common adhesives or fixatives used to fix tissue sections to slides. No difficulty was experienced with cracking, peeling or slipping-off of films when carob bean gum solution was used and the above staining procedure followed and slides rinsed gently in a container of water.

Numerous trials indicated that 1 g. of butter in 9 ml. gum solution and 0.1 ml. of this on a slide provided about the optimum dilution. A greater amount of butter increased the difficulty of fat removal and too small a sample provided too little mold for counting.

A number of common staining techniques were tried, including the Gram stain, negative staining, and staining with methylene blue after defatting in xylol. None of these showed any advantage over the simple Newman method used for milk smears. Substitution of half of the methylene blue in the Newman stain with basic fuchsin resulted in a preparation that stained the mold more intensely on some of the slides. A better staining technique for this purpose can possibly be developed.

For a sample of high mold content 25 fields per slide should be sufficient to count; however, 50 fields is preferable for a sample with moderate or low mold content. On the basis of present official standards for butter, 20 mm. per mgm. would be considered a high count. Duplicate slides should be prepared and results averaged.

RESULTS

A number of trials were carried out to determine the reliability of the new method. These included checks between various slides from the same sample, different samples from the same print of butter and counts on the same prints by two technicians using different microscopes with separate preparation of samples and slides.

TABLE 1
Results on different samples from the same prints of butter

Mm. mold per mgm. butter					
Sample A			Sample B		
Slide 1	Slide 2	Ave.	Slide 1	Slide 2	Ave.
12.87	11.20	12.04	10.19	11.85	11.02
3.80	3.43	3.62	3.80	3.52	3.66
8.15	9.72	8.94	12.13	8.89	10.51
2.87	3.89	3.38	3.33	2.96	3.15
20.46	19.54	20.00	19.08	18.06	18.57
25.74	23.15	24.45	25.56	25.09	25.33

TABLE 2
Counts with new method by two operators using different microscopes

Sample No.	Total combined length of mold fragments	
	A	B
	<i>mm. per mgm.</i>	<i>mm. per mgm.</i>
320	16.56	16.56
321	48.85	48.76
322	21.25	25.76
323	7.82	8.10
324	16.74	20.24
325	14.17	12.70
326	21.16	22.45
327	11.87	10.76

The values shown in table 1 are from a trial to determine the consistency of results between duplicate samples. As the results indicate, with one exception, the variation between the four slides on the two samples is relatively small for a microbiological analysis. The mold content of different portions of a print of butter appeared fairly constant. Whether different portions of a commercial churning would check as well with this method has not been determined. The results of Claydon (2) indicate that some significant variations may occur due to chance variation of mold filaments in different portions of a churning.

Two different operators employing different microscopes and separate preparation of samples can obtain comparable results with the new method (table 2). The two technicians making these counts had received only a few hours training in appearance and detection of mold hyphae in butter. Again the variations occurring are no greater than would be expected in a microbiological analysis.

Comparisons between values with the Wildman and new methods are too few to enable definite conclusions, but those presented in tables 3 and 4 at least indicate in a general way what might be expected. There appears to be a rough correlation between the results until the mm. per mgm. reach

TABLE 3
Comparative values with new method and Wildman method on one series of commercial butter samples

Sample No.	New method	Wildman method
	<i>mm. per mgm.</i>	<i>per cent positive fields</i>
66	0.88	2
296	3.26	12
294	3.64	16
295	9.72	46
293	11.53	44
297	19.29	80
298	24.89	96
299	49.54	92
300	75.01	96

about 25, after which the Wildman method shows little increase. It should be emphasized that further comparisons in large number must be made before any definite correlation between the two methods can be worked out. However, it appears from these preliminary results that when the mold content reaches about 15 mm. per mgm., a sample runs a good chance of having a Wildman count of 60, and when the mold content reaches 20 mm. per mgm., a sample will usually be definitely illegal. At about 25 mm. per mgm. the Wildman count will vary from 75-100 per cent positive fields and remain at about this figure as the mm. per mgm. increase. Commercial butters with more than 40 mm. per mgm. are not uncommon.

Two samples with the same total length of mold filament per mgm. may exhibit considerably different Wildman values. This is demonstrated best by two samples one of which contained 12.84 mm. per mgm. total mold

TABLE 4

Comparative values obtained on commercial butter samples with new method and Wildman method

Number of samples in group	New method	Average count by Wildman method	Range of Wildman counts
	<i>mm. per mgm.</i>	<i>per cent positive fields</i>	<i>per cent positive fields</i>
9	1- 5	10	0-16
5	5-10	33	12-46
12	10-15	49	32-68
9	15-20	69	49-80
8	20-25	80	64-96
3	25-35	87	76-96
5	35-50	93	88-96
1	75.01	96

and a Wildman count of 32 and the second 12.21 mm. per mgm. and 68 per cent Wildman count. Similarly two samples with about 16 mm. per mgm. total mold, gave Wildman counts of 49 and 80 respectively.

DISCUSSION

The success in duplicating results from one sample or operator to the next emphasizes the quantitative nature of the new method. In order for the method to be actually quantitative and consistent, however, it is necessary that the operator be thoroughly familiar with microscopic appearance of mold fragments and also make absolutely certain that he is accurately estimating lengths of mold fragments in terms of mm. The ruled ocular disc employed for the Howard or Wildman counts provides sufficient rulings to enable a reasonably accurate estimation of total combined length of mold fragments in every field. Once the operator has become accustomed to estimating length of filaments, the method becomes almost as rapid as that of Wildman.

The method as it exists has some advantages and disadvantages. It does provide a truer picture of the actual mold hyphae content than any other method. The results of Claydon indicate that the degree of working in the churn affects the number and length of mold filaments in the final butter and consequently the Wildman mold mycelia value. By the new method all filaments are included whether long or short. Neither the Wildman nor the new method includes the tiniest fragments of *Oospora lactis* hyphae sometimes referred to as spores. However, examination indicated that only a comparatively small number of the tiny fragments remain in the butter. Most of them pass out into buttermilk and wash water.

There is a definite advantage to knowing the actual total mold content of a sample. The commercial samples in these studies running as high as 49 and 75 mm. per mgm. total mold filament provide an example of a problem confronting many creameries. The high temperatures and relative humidities in this part of the country are quite conducive to mold growth in cream on the farm. Creameries in this area are therefore blessed with an abundance of mold growth in the cream during the warm season. The result is that much 90 score butter runs over 60 per cent by the Wildman test. The 89 score butter is often correspondingly higher in mold content. Much of it runs as high as 90 to 100 by the Wildman test. Such butter may contain as much as 75 mm. per mgm. of mold by the new method. If the creameryman in a quality improvement program eliminates half the moldy cream, he may bring the mold content of his butter down to about 40-50 mm. Yet it still will run 90 or over by the Wildman test. If he then again eliminates 50 per cent of this moldy cream, he reduces the mold content of his butter to perhaps 25 mm. per mgm. Again instead of reducing the Wildman count 50 per cent he may end up with a 90 to 100 per cent mold mycelia count. It is not until the mold content is reduced below 20-25 mm. per mgm. that a distinct lowering in mold content of butter can be detected by the Wildman mold mycelia count. This condition has been responsible for considerable misunderstanding in cream quality improvement work.

The variation in Wildman mold mycelia count values for butters of equivalent total mold content indicates that more study should be given to factors possibly responsible for such variations. A variation of 20 or 25 per cent due possibly to manufacturing conditions may easily throw a butter from a definitely legal into an illegal class.

Another advantage of the new method is that any microscope of any field diameter can be employed as long as it provides about 100 \times magnification and a stage micrometer is available to determine the field diameter. Also, a permanent preparation of the sample is obtained which can be examined weeks later if more convenient. Materials other than butter can be diluted in hot gum solution and their total content of mold filament determined. Because of the interfering effect of the protein, samples of cream and butter-

milk must be diluted out further than butter. Because of its low mold content, butter wash water must be diluted less than butter. Covering the preparation with immersion oil is especially helpful in examining cream and buttermilk preparations. In cream examination, special methods are necessary to thoroughly agitate the samples in order to break up masses of mold filaments.

This method was not developed nor is it presented for the purpose of replacing the Wildman count. It may appear too technical for the average control laboratory. However, it has been about as easy to teach to a careful operator as the Wildman method. The new method, because it is quantitative, has proven a valuable research tool. Investigations on mold content of butter and other materials have been carried out that would not have been possible by any other method.

SUMMARY

A method for determination of total combined length of dead mold fragments per gram or milligram of butter has been developed.

The procedure consists of dilution of one gram of butter in hot gum solution, spreading a small quantity of this on a glass slide, staining with Newman's stain and examining with low power magnification. The average length of mold filament per field is multiplied by the microscope factor divided by 1000 and results reported in mm. per mgm. of butter.

This quantitative method has been of value in studies to determine the total mold fragment content of butter, buttermilk, wash water and cream.

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THE DETERMINATION OF CITRIC ACID IN MILK PRODUCTS BY CERATE OXIDIMETRY

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Of the various methods which have been proposed for the determination of citric acid in milk, those based on Stahre's (12) reaction have come into wide use. In this method, the citric acid is oxidized by dilute potassium permanganate in the presence of bromine resulting in the formation of insoluble pentabromacetone, which is determined gravimetrically.

Allen (1) points out three objections to this method: "1), the formation of pentabromacetone as an oil instead of a crystalline precipitate; 2), the relatively high solubility of the precipitate; 3), the loss due to volatility when moist."

By determining the solubility of the precipitate (and using a factor to include this loss in the final calculations) and by drying the precipitate in a vacuum at less than 20° C. for sixteen hours, Deysher and Holm (2) largely eliminated the second and third errors pointed out by Allen. But in so doing, the length of time of performing the test was extended another twenty-four hours, making a total length of time of three days.

If the determination of citric acid is to be used as a control test in the manufacture of dairy products, particularly evaporated milk, a much shorter method is necessary.

A search of literature, therefore, was made to ascertain if other methods would be applicable. The conductimetric method of Kolthoff (6) was tried but found to be unsuitable for milk owing to the high concentration of other ions, particularly chloride. Potentiometric titrations using silver nitrate, barium acetate, and lead acetate resulted in failures because of interfering phosphate and chloride ions. Rundell (9) states that attempts to determine citric acid potentiometrically with calcium chloride were unsuccessful.

The iodimetric method of Kometianni (7) involves precipitation of the citric acid as pentabromacetone under conditions which are not in accordance with those found necessary by Deysher and Holm for quantitative recovery. This is in agreement with Templeton's (13) statement that the iodimetric method is not quantitative. Hartman (3) also states that Kometianni's procedure is not entirely satisfactory when applied to mixed fruit acids. If the method were modified to insure accuracy, it would offer no advantage over the Deysher and Holm procedure.

Colorimetric methods in which acetic anhydride and pyridine are used to develop color directly from the citrate ion were found to be unsatisfactory, probably due to the presence of the chloride ion. Methods using

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acetone dicarboxylic acid or pentabromacetone for a basis for color development were not tried since no great saving in time would have resulted.

Willard and Young (14) showed that ceric sulphate in sulphuric acid solutions oxidized citric acid at the rate of 1.211 mg. anhydrous citric acid per 1 ml. of 0.1 N sulphato-cerate. Smith and Duke (10) found that the perchlorato-cerate ion in the presence of perchloric acid oxidized citric acid stoichiometrically at the rate of 1.372 mg. per 1 ml. of 0.1 N perchlorato-cerate. When these two methods were applied to milk, it was found that the former method was not stoichiometric. The following method is based on Hartman and Hillig's procedure (4, 5) for isolating the citric acid (omitting tartaric and phosphotungstic acids which interfere) and Smith and Duke's procedure for oxidizing it. In preliminary work, to determine the adaptability of their indicator to the method as applied to milk, a Beckman pH set was employed using a glass electrode as reference electrode and polished platinum wire as indicating electrode following the suggestion of Lykken (8).

PREPARATION OF REAGENTS

1. *Ammonium perchlorato-cerate.* Smith (11). Dissolve 55 to 56 grams of $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ by adding the salt to a liter beaker containing 340 to 345 ml. of 72 per cent perchloric acid. Stir well during a half-minute interval and add 100 ml. of water. Again stir well for a half-minute interval and add an additional 100 ml. of water. Continue this procedure until the volume attains 1 liter. Transfer to a suitable glass-stoppered reagent bottle and store in the dark. It is preferable to store the perchlorato-cerate solution in a black bottle, conveniently made by completely covering a reagent bottle with black electrical insulating tape. Such a solution requires standardization about every fifteen days. Carbon or dust if allowed to come in contact with perchlorato-cerate solutions catalyze their slight deterioration rate. The perchlorato-cerate solution is standardized by measuring 20.00 ml. into a 150 ml. beaker and adding 15 ml. of 70 per cent perchloric acid, 35 ml. of water, and 1 drop of nitro-ferroin. Titrate with 0.1 N sodium oxalate to the first formation of a pink color.

2. *Sodium oxalate in 0.1 N perchloric acid.* Smith (11). Dissolve 6.706 grams of Bureau of Standards, standard of reference sodium oxalate, per liter of 0.1 N perchloric acid. This solution is stable upon storage. It is used to standardize the ammonium perchlorato-cerate solution and to titrate the excess perchlorato-cerate left after oxidation of the citric acid.

3. *Nitro-ferroin* (nitro-o-phenanthroline ferrous complex) used as internal indicator is commercially available.

4. *Lead acetate.* Dissolve 75 gms. of normal lead acetate in water, add 1 ml. of glacial acetic acid and dilute to 250 ml.

5. *Blank solution.* Dissolve 0.5 gm. lactose, 0.2 gm. CaCl_2 , and 0.2 gm. KH_2PO_4 in 100 ml. H_2O .

6. *Saturated lead citrate.* Add a slight excess of the lead acetate solution to a 10 per cent solution of A.C.S. grade citric acid in distilled water. Wash several times with distilled water and allow to stand at least twenty-four hours. Filter through No. 42 Whatman or similar grade filter paper before using. (At 25° C., lead citrate is soluble to the extent of 6 mg. per 100 ml. of water.)

PROCEDURE

Weigh 50 grams of whole milk, 25 grams of evaporated milk, or 5 grams of milk powder in a 150-ml. beaker. Add 25 ml. of water to the evaporated milk and 45 ml. to the dried milk. Add 6 ml. of 1 N sulphuric acid and heat for fifteen minutes on a steam bath. Cool. Add 95 per cent ethyl alcohol and transfer to a 250-ml. volumetric flask. Make to 250 ml. with alcohol. Mix and filter through a good grade of filter paper (such as Whatman No. 1). If filtrate is not clear, repeat the filtration. Place 100.0 ml. of the filtrate in a 100-ml. centrifuge tube and add 4 ml. of lead acetate solution. Allow to stand 3–5 minutes with occasional shaking. Centrifuge 5 minutes. Decant, testing the alcoholic solution with a few drops of lead acetate to insure complete recovery of lead citrate. Drain for 5 minutes. Add 30 ml. of water saturated with lead citrate. Shake until precipitate is dislodged, centrifuge 5 minutes, decant, and drain for 5 minutes. Wash 2 more times with 20-ml. portions of water saturated with lead citrate, centrifuging and draining 5 minutes each time. Add 10 ml. water and shake to dislodge the precipitate. Transfer to a 250-ml. beaker using a total of 55 ml. of 70 per cent perchloric acid to wash the centrifuge tube, pouring the washings into the beaker. Place beaker in ice and salt mixture until temperature reaches –3° to 0° C. Add 40.0 ml. 0.1 N perchlorato-cerate slowly with stirring, not allowing the temperature in the beaker to rise above 10° C. Hold for 30 minutes at 10° C. Add 95 ml. water, 2 drops of nitro-ferroin and titrate with 0.1 N sodium oxalate.

A blank determination must be run with each group as follows:

Place 25 ml. of the prepared blank solution in a 150 ml. beaker. Add 3 ml. H₂SO₄. Heat in steam bath with other samples. Cool. Add 95 per cent alcohol and make to 125 ml. Mix and filter. Continue exactly as described for milk, except that at the conclusion of the final draining period, 30 ml. of water are added and the contents of the centrifuge tube transferred to a 250-ml. beaker with 55 ml. 70 per cent perchloric acid. When the temperature has reached –3° to 0° C., 10.00 ml. of 0.1 N perchlorato-cerate are added and the solution held for 30 minutes at 10° C. Add 95 ml. water, 2 drops of nitro-ferroin and titrate with 0.1 N oxalate. The ml. of 0.1 N cerate used constitute the blank.

Per cent citric acid (anhydrous)

$$= \frac{\text{ml. 0.1N perchlorato-cerate used} - \text{blank} \times 0.343}{\text{wt. sample}}$$

TABLE 1
Recovery of citric acid from pure solutions*

Citric acid taken	Recovered	Recovered
mg.	mg.	%
33.51	33.68	100.52
33.51	33.23	99.17
33.51	33.71	100.59
33.57	33.57	100.00
33.57	33.63	100.18
33.57	33.42	99.55

* 1 ml. 0.1 N perchlorato-cerate is equivalent to 1.372 mg. anhydrous citric acid.

The potentiometric method for the detection of the endpoint is equally as satisfactory as the use of nitro-ferroin as an internal indicator. The results included in this paper were secured with the nitro-ferroin indicator.

EXPERIMENTAL

Various quantities of anhydrous citric acid (prepared by grinding to a powder and drying Baker's analyzed and Merck's A.C.S. grade over calcium chloride) were dissolved in distilled water. Sufficient 70 per cent perchloric acid was added to make the solution 4 M with respect to the perchloric acid. After cooling the mixture to 0° C., a slight excess of 0.1 N perchlorato-cerate was added and the solution held at 10° C. for 30 minutes. It was then diluted to 2 N and titrated with 0.1 N sodium oxalate using nitro-ferroin as the indicator. The results given in table 1 show an average recovery of 100.00 per cent. It was found that if the temperature rose above 12° C., the results were high owing to side reactions as pointed out by Smith (11).

Four cultures of citric acid fermenting bacteria were secured from Iowa State College. These were *S. diacetylactis* strain N 4, and *S. citravorous* strains S 9, S 14, and Mu 29. These were inoculated into acidified sterile skim milk and incubated until citric acid was absent as shown by the pentabromacetone determination. Volumetric citrate determinations were made

TABLE 2
Recovery of citric acid from decitrated milk

Citric acid taken	Recovered	Recovered
mg.	mg.	%
15.1	15.1	100.0
22.2	22.8	102.7
42.4	43.3	102.1
45.4	46.0	101.3
69.3	70.0	101.0
90.0	90.1	100.1
98.9	99.0	100.1
98.9	98.8	99.9

on these cultures with known quantities of anhydrous citric acid added. These results are given in table 2. The average percentage recovery is 100.9 per cent.

It was found that decitrated milk gave a larger blank value than when the procedure was followed using only distilled water. It was thought that this might be due to some interfering substance in milk or to the formation of some interfering substance during the growth of the citric-acid-fermenting bacteria. Various natural components of milk were tested, including lactose, riboflavin, niacin, ascorbic acid, calcium and magnesium salts, phosphates, chlorides, nicotinic acid, pantothenic acid, lactic acid, propionic acid, butyric acid, acetylmethylcarbinol, and ether extract from milk but none of these was found to interfere. It was found that when the volume of the precipitate

TABLE 3

Method of preparation of blank	ml. of 0.1 N perchlorato-cerate required per 100 ml. filtrate
Fermentation of citric acid	2.35
	2.45
	3.11
	2.38
	3.27
	2.88
Precipitation of proteins and citrate with silver acetate and acetic acid	2.60
	2.94
	2.68
	1.57
Prepared blank (containing lactose, Ca, Cl, PO ₄)	2.10
	2.24
	3.35
	2.87
	3.35

was the same as that obtained from milk the blank was about the same. Since repeated washings of the precipitate did not appreciably reduce the value of the blank it was thought that possibly a small amount of the lactose was occluded in the precipitation of lead citrate. However, when the thrice washed precipitate was tested for lactose using copper sulphate and alkaline tartrate solutions, no observable precipitate of copper oxide was formed. Furthermore, when perchloric acid is added to the washed precipitate of lead salts, both in the case of the decitrated milk and in the case of the various milk products examined, a yellow color is formed which does not form with any of the substances added to the blank. It is apparent, therefore, that some substances not included in this study is present in the lead salt precipitate which may interfere.

Another method for the preparation of the blank was tried, using 0.5 gm. silver acetate and 5 ml. of 10 per cent acetic acid per 50 ml. of milk in place of 6 ml. NH₂SO₄. By this procedure the citrate is precipitated as silver

citrate and filtered off. To 100 ml. of the filtrate, 4 ml. of lead acetate were added and the precipitate washed as previously described. This precipitate did not give the color reaction with perchloric acid and the blanks were slightly lower than those from the decitrated milk. However, the volume of the precipitated lead salts is small. A resume of these results is given in table 3.

Table 4 gives results of some analyses of powdered skim milk, whole milk, and evaporated milk. The volumetric citrate method gives results which are slightly higher due either to an interfering substance in milk or incomplete recovery by the pentabromacetone method.

TABLE 4

Product		Oxidation by perchlorato-cerate	Pentabromacetone (Modification of Deysher and Holm)
Powdered milk:	(1)	1.57	1.55
		1.57	1.52
	(2)	1.82	1.67
		1.87	1.63
	(3)	1.82	1.78
		1.80	1.74
Whole milk:	(1)	0.172	0.161
		0.176	0.168
	(2)	0.178	0.159
		0.168	0.167
	(3)	0.171	0.165
		0.171	0.160
Evaporated milk:	(1)	0.332	0.323
		0.325	0.327
	(2)	0.334	0.330
		0.340	0.333
	(3)	0.342	0.329
		0.333	0.338

CONCLUSIONS

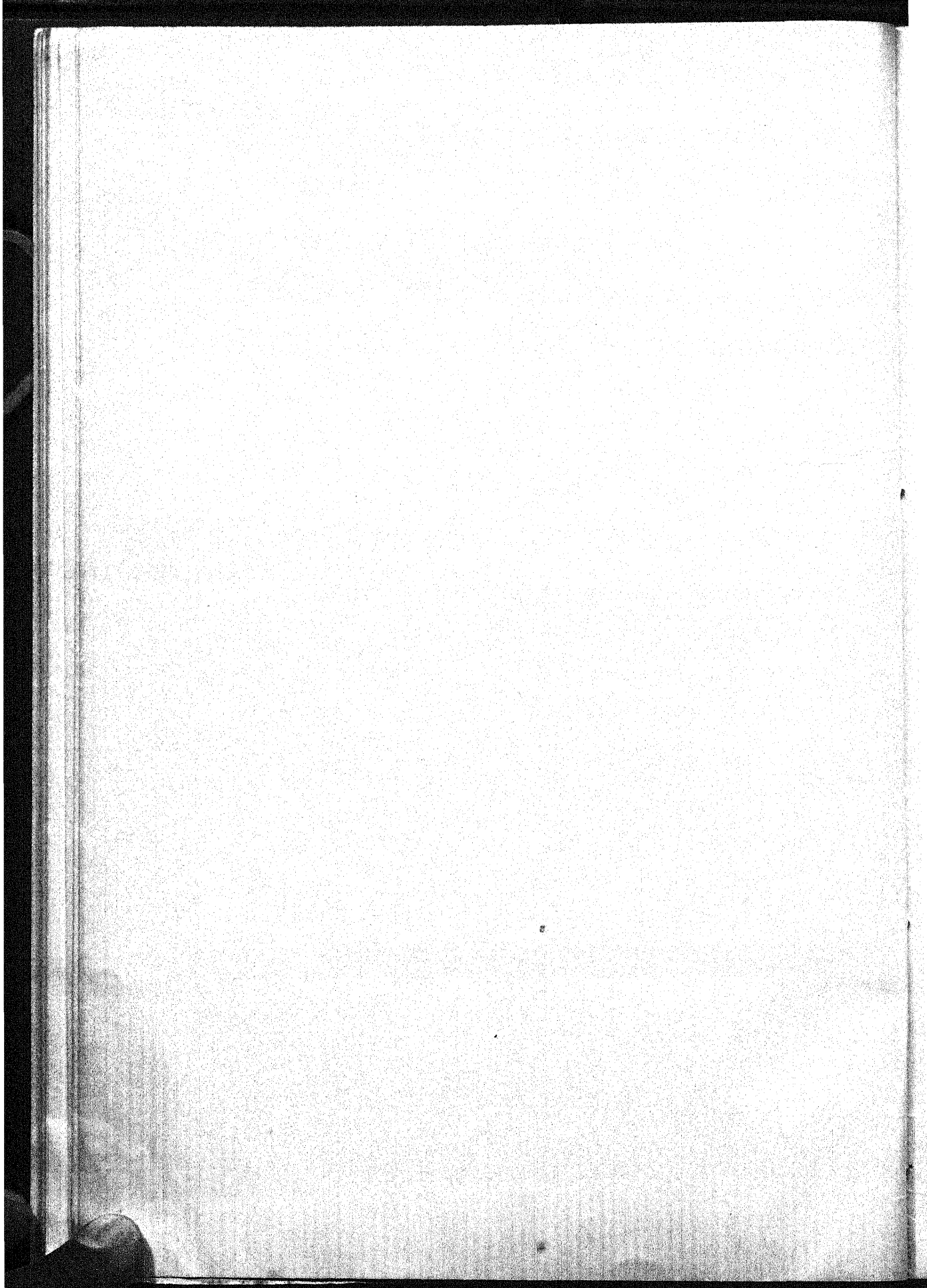
A method for the determination of citric acid in milk products is presented which is based on the oxidation of citric acid (isolated from interfering substances by precipitation as lead citrate) by an excess of 0.1 N perchlorato-cerate in 4 M perchloric acid solution. The excess perchlorato-cerate is titrated with 0.1 N sodium-oxalate in 2 M perchloric acid using nitro-ferroin as an internal indicator.

Care must be used to free the citric acid from other organic substances which are also oxidized by the perchlorato-cerate. The results from the method are slightly higher than those from the pentabromacetone method and can be secured in considerably less time.

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THE EFFECT OF IODINATED CASEIN (PROTAMONE) ON MILK AND BUTTERFAT PRODUCTION AND ON THE ASCORBIC ACID CONTENT OF THE MILK*

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In 1934, Graham (3) reported the results of extensive studies with thyroidectomized dairy cattle and with the feeding of desiccated thyroid glands to lactating cows. In this experiment the diminution in milk secretion following the removal of the thyroid could not be distinguished readily from that accompanying a control operation without the removal of the thyroid gland; however, there was a distinct rise in milk secretion, when small amounts of dried thyroid glands were fed either to thyroidectomized cows or to unoperated normal cows in the declining phases of lactation. The stimulating factor in the thyroid glands was shown to be thyroxine by an increase in milk secretion when synthetic thyroxine was injected (4). These observations have since been confirmed and extended by Jack and Beechdel (7), Folley and White (2), and Herman, Graham and Turner (5).

The practical value of feeding pure thyroxine or desiccated thyroid glands to increase milk production was largely nullified by the exorbitant cost of this material. In 1940, Turner (13) reported a possible cheap source of material with thyroxine activity in the form of iodinated casein. Reineke and Turner (10) have recently described a method for the preparation and the use of this synthetic thyroprotein. This material is prepared by the iodination of casein in solution to which sufficient sodium bicarbonate has been added to maintain the pH of the reaction mixture within the range of 6.8 to 8.0.

The administration of this artificial thyroprotein to cows and goats, according to Reineke and Turner (11), stimulates increases in milk production and the percentage of fat in the milk similar in every respect to those produced by either desiccated thyroid glands or synthetic thyroxine.

The purpose of this paper is to report the results obtained with lactating dairy cows in the declining part of the lactation period, when the iodinated casein was fed in smaller quantities but for longer periods of time than that reported previously by Reineke and Turner (11).

EXPERIMENTAL

Six pure-bred Holstein cows in the sixth to the eighth month of their first lactation were selected from the regular milking herd for this experiment.

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The cows were producing from 30 to 40 pounds of milk per day at the beginning of the experiment. The experiment was a double reversal feeding trial started on the twenty-first day of February and continued for a period of 13 weeks or until May 22. The first week was used as an adjustment period at which time the cows were divided into two equal groups of three cows each on the basis of milk production, fat production, and body weight.

Group 1, composed of cows number 535, 536, and 544 was fed 15 grams of iodinated casein (Protamone) per cow daily as a supplement to the regular ration for the first 4-week period. The iodinated casein was discontinued for the second 4-week period after which it was again fed during the third 4-week period.

Group 2, consisting of cows number 527, 529, and 541, served as a check for the first 4-week period and then was fed 15 grams of the iodinated casein per cow daily for the second 4-week period, after which it was discontinued for the last or the third 4-week period.

During the first two 4-week periods, the cows were confined to the barn except when they were allowed to run outside in a clean lot for exercise. During the last 4-week period of the experiment the cows were on pasture. The ration fed the cows in each group was composed of a concentrate mixture, legume hay and beet pulp. The cows were fed daily 10 pounds of concentrates, 2 pounds beet pulp and all the hay they would clean up. They were fed a uniform amount of feed throughout the experiment regardless of the amount of milk produced and the gain or loss in body weight.

The cows were milked twice daily. On Monday, Wednesday, and Friday of each week they were weighed and one-half pint samples of milk were collected night and morning for analysis. Each sample of milk was analyzed for fat by the Babcock method and for total solids by the oven method. Ascorbic acid was titrated in the samples collected each morning with 2-6 dichlorophenolindophenol as described by Sharp (12).

Close observations were made each day for any unusual reaction which might become apparent. A maximum-minimum thermometer placed in the barn was read daily in order to note any unusual change in temperature. Respiration and pulse rates were obtained between 2 and 4 P.M. on Friday of each week during the first two 4-week periods when the cows were confined to the barn. The pulse was counted by feeling the posterior tibial artery on the medial surface of the tibia at a point eight to ten inches above the hock joint.

RESULTS

Data showing the effect of feeding iodinated casein on milk and butterfat production; the percentage fat and solids-not-fat; the ascorbic acid in the milk and changes in body weight are presented in table 1 and in figures 1 to 6. Data for individual days were averaged and presented as weekly averages for individual animals or as groups as the case might be. The

figures represent averages for the separate groups except in the case of figure 4, which shows the effect of iodinated casein on solids-not-fat and where variation in the results obtained made it seem desirable to present data for individual animals. It may be observed in table 1, that data for 529 are incomplete. This animal appeared normal until about the middle of the second 4-week feeding period. She had received 15 grams of the iodinated casein daily for two weeks when she began to show the symptoms of a rabid animal. She refused to eat and lost in body weight rapidly, and died in about a week after showing the first symptoms. After an examination of the brain, the cause of the death of the animal was definitely diagnosed as rabies. Data from this animal are not included in the averages for Group 2.

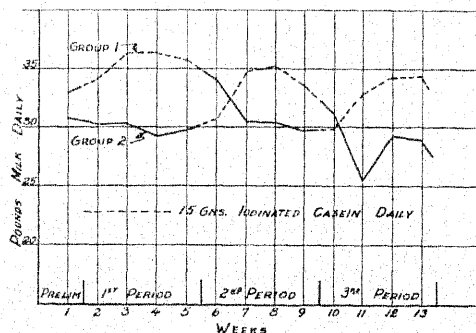


FIG. 1. Effect of iodinated casein on milk production.

Effect on milk production. Data presented in table 1 and in figure 1 show that the feeding of 15 grams of iodinated casein daily, as a supplement to the regular ration, did cause a definite increase in the milk production of all animals on experiment. It may be observed in figure 1 that the cows increased in milk production for about two or three weeks after the beginning of iodinated casein feeding, after which there was a tendency to show a slight downward trend. It may be observed in the case of the cows in Group 2 that milk production showed a sharp decline for about two weeks during the third period when iodinated casein had been discontinued. During the latter part of the third period there was an increase in milk production which is thought to be due to the cows going out on pasture. At that particular time the cows in Group 1 were receiving iodinated casein which probably supplemented the stimulating effect of pasture.

Effect on percentage fat content of the milk. Data summarized in figure 2, show the effect of feeding iodinated casein on the fat content of the milk. The cows in both groups showed an increase in percentage fat content of the milk during the feeding periods. It may be observed that there was considerable carry-over effect following the feeding of the iodinated casein. After the feeding of the iodinated casein, the cows continued to secrete milk

TABLE 1
Average daily milk and fat production, the percentage fat, and solids-not-fat and the ascorbic acid content of milk

Weeks	Date	Milk, lbs.	Fat, %	Fat, lbs.	S-N-P, %	Ascorbic acid, mg./liter	Milk, lbs.	Fat, %	Fat, lbs.	S-N-P, %	Ascorbic acid, mg./liter	Milk, lbs.	Fat, %	Fat, lbs.	S-N-P, %	Ascorbic acid, mg./liter
Group I																
15 grams of iodinated casein daily																
1	Feb. 21-27	37.9	3.83	1.42	8.53	20.2	32.8	4.07	1.33	9.00	18.4	29.1	3.62	1.05	8.41	16.7
2	Feb. 28-Mar. 6	35.8	3.81	1.36	8.50	19.2	34.6	4.13	1.43	9.03	15.3	32.0	3.83	1.23	8.56	14.4
3	Mar. 7-13	38.8	4.07	1.58	8.87	16.2	36.8	4.42	1.63	9.00	12.9	33.7	3.99	1.34	8.78	19.9
4	Mar. 14-20	39.0	4.30	1.68	8.77	15.2	36.0	4.51	1.62	8.99	11.9	34.2	4.20	1.44	8.78	9.9
5	Mar. 21-27	38.7	4.22	1.63	8.75	15.7	35.7	4.68	1.67	8.92	12.3	33.0	4.41	1.46	8.69	11.5
Iodinated casein discontinued																
6	Mar. 28-Apr. 3	37.0	4.14	1.53	8.93	17.8	33.3	4.68	1.56	9.12	14.3	31.9	4.11	1.31	8.74	14.2
7	Apr. 4-10	34.3	4.58	1.57	8.50	17.6	28.6	4.65	1.33	9.12	15.9	28.7	4.07	1.17	8.94	15.4
8	Apr. 11-17	34.2	3.79	1.30	8.64	16.2	29.5	4.81	1.42	9.10	13.4	27.5	3.95	1.09	8.80	13.0
9	Apr. 18-24	31.6	4.15	1.31	8.43	17.3	28.9	4.41	1.27	9.23	15.3	28.9	3.90	1.13	8.74	15.1
15 grams of iodinated casein daily, and cows on pasture																
10	Apr. 25-May 1	32.1	4.00	1.28	8.56	15.7	28.1	4.39	1.23	9.15	13.6	29.4	3.93	1.16	8.71	10.7
11	May 2-8	34.7	4.55	1.58	8.43	12.9	33.4	4.67	1.56	9.07	10.7	30.2	4.48	1.35	8.67	8.8
12	May 9-15	37.0	4.73	1.75	8.35	13.9	34.9	6.10	2.13	9.19	11.3	30.9	5.10	1.58	8.57	9.3
13	May 16-22	37.4	4.59	1.72	8.34	15.7	32.3	5.37	1.73	9.25	12.4	33.2	4.58	1.52	8.76	10.9

TABLE 1—(Continued)

Weeks	Date	Milk, lbs.	Fat, %	Fat, lbs.	S-N-P, %	Ascorbic acid, mg./liter	Milk, lbs.	Fat, %	Fat, lbs.	S-N-P, %	Ascorbic acid, mg./liter	Milk, lbs.	Fat, %	Fat, lbs.	S-N-P, %	Ascorbic acid, mg./liter
Group II																
15 grams of iodinated casein daily																
		Cow Number 527					Cow Number 529					Cow Number 541				
1	Feb. 21-27	34.6	3.77	1.30	8.36	17.2	33.0	3.87	1.28	8.53	19.7	27.0	3.25	0.88	8.51	19.0
2	Feb. 28-Mar. 6	34.4	3.88	1.33	8.30	17.0	31.1	3.78	1.18	8.63	19.5	25.9	3.76	0.97	8.41	19.9
3	Mar. 7-13	34.4	3.77	1.30	8.33	17.7	32.9	3.77	1.24	8.62	19.4	26.1	3.52	0.92	8.56	19.3
4	Mar. 14-20	32.6	3.83	1.25	8.15	15.9	31.1	3.98	1.24	8.60	19.0	25.9	3.52	0.91	8.45	18.0
5	Mar. 21-27	33.8	3.85	1.30	8.25	15.8	30.2	4.03	1.22	8.69	18.7	25.7	3.67	0.94	8.55	17.7
Iodinated casein discontinued, cows on pasture																
6	Mar. 28-Apr. 3	34.4	3.81	1.31	8.17	12.7	32.9	3.69	1.21	8.68	11.9	27.0	3.46	0.93	8.72	16.6
7	Apr. 4-10	38.6	4.32	1.67	8.39	9.6	35.3	3.50	1.24	8.77	13.8	31.1	3.60	1.12	8.64	12.1
8	Apr. 11-17	39.1	4.21	1.65	8.99	8.6	31.3	3.97	1.24	9.10	12.2
9	Apr. 18-24	36.3	4.19	1.52	8.76	10.0	31.1	4.32	1.41	8.89	13.5
10	Apr. 25-May 1	32.8	4.12	1.35	8.89	13.3	29.4	3.93	1.16	8.94	13.2
11	May 2-8	27.3	4.64	1.27	8.67	15.5	23.3	4.34	1.01	8.81	16.2
12	May 9-15	32.4	4.32	1.40	8.35	13.1	26.2	4.71	1.23	8.39	14.4
13	May 16-22	31.7	4.06	1.29	8.39	14.1	26.2	4.28	1.12	8.33	12.8

* Cow died with rabies.

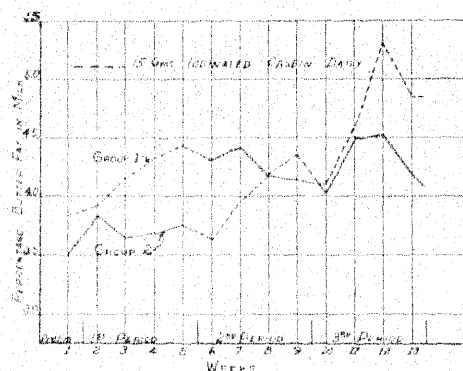


FIG. 2. Effect of iodinated casein on percentage butterfat in milk.

of a higher fat content than before, for a period of at least four weeks. In the case of the cows in Group 1, there was an increase from an average of about 3.8 per cent to about 4.4 at the end of the first 4-week feeding period. During the next 4-week period when the cows did not receive the iodinated casein the percentage fat decreased only about 0.2 per cent. During the last 4-week feeding period when the cows were again fed iodinated casein, the fat percentage increased to about 5 per cent. This represents about 1.2 per cent increase in the fat content of the milk during the experiment. A small part of this increase in fat percentage may have been due to advance in lactation.

The cows in Group 2 showed an increase in the percentage fat from about 3.7 to about 4.4 per cent after four weeks of casein feeding. They were able to maintain a level of about 4.4 per cent for the following four weeks when they received no iodinated casein.

Effect on total fat production. Data summarized in figure 3, show the effect of iodinated casein on total fat production. In general butterfat pro-

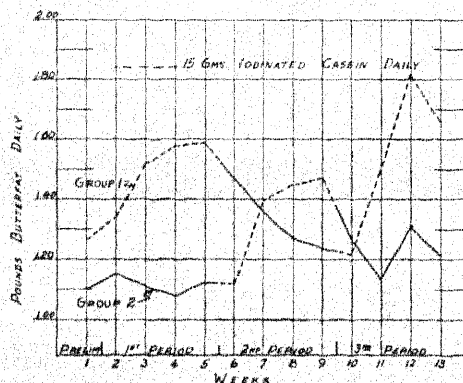


FIG. 3. Effect of iodinated casein on butterfat production.

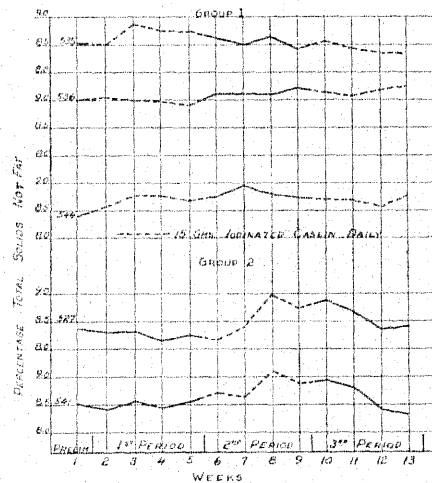


FIG. 4. Effect of iodinated casein on the percentage total solids-not-fat of milk.

duction followed about the same trend as milk production for the animals in both groups. The relative change in fat production due to casein feeding was considerably greater than in the case of milk production. This was brought about by both an increase in milk production and an increase in the percentage fat content of the milk.

Effect on the solids-not-fat content of the milk. In table 1 and in figure 4 are presented data for the solids-not-fat content of the milk for the animals in both groups. It may be observed that the changes in the solids-not-fat content are small, but there seems to be a tendency for a small increase for animals Number 535 and 544 during the first iodinated casein feeding periods, with a slight decrease during the next period for Number 535 when she did not receive iodinated casein. In the case of cows Number 527 and

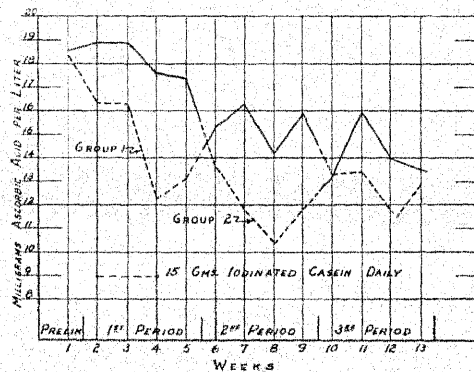


FIG. 5. Effect of iodinated casein on ascorbic acid content of milk.

541, there seems to be a much more definite tendency for the solids-not-fat to show an increase during the iodinated casein feeding with a decrease during the next 4-week period when the cows did not receive any iodinated casein.

Effect on the ascorbic acid content of the milk. In figure 5 is presented a summary of the data presented in table 1, showing the effect of feeding iodinated casein on the ascorbic acid content of the milk. It may be observed that there was a definite decrease in the ascorbic acid content of the milk in all cases when iodinated casein was fed. When the iodinated casein feeding was discontinued there was an increase in the ascorbic acid in the milk, but there seemed to be some carry-over effect. It may be observed that the general trend was downward as the experiment progressed.

Effect on change in body weight of the cows. In figure 6 is presented a summary of the changes in body weight of the cows in both groups. It may

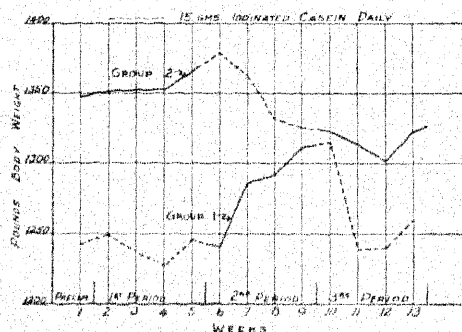


FIG. 6. Effect of iodinated casein on body weight.

be noted that the cows in Group 1 did not show any particular change in body weight during the first iodinated casein feeding period but showed an increase of about seventy pounds during the second 4-week period when iodinated casein was not fed. During the third period when they received iodinated casein they showed a decrease in body weight which was about equal to the gain made during the preceding period.

The cows in Group 2 showed a tendency to gain slightly in body weight during the first 4-week period when not receiving iodinated casein but declined considerably during the second 4-week period when they received iodinated casein. They continued to show a decline in body weight for about two weeks during the third period when they were not receiving iodinated casein. After the middle of the third period body weight began showing an upward trend when they were turned to pasture.

Effect on pulse rate and respiration. In table 2 are presented data showing the effect of iodinated casein on the pulse and respiration rates. Data are also presented showing the minimum and maximum barn temperatures on the days that the pulse and respiration rates were determined.

TABLE 2
Effect of iodinated casein on the pulse rate and respiration

Group 1										Group 2						Barn tem- perature, °F.			
535				536		544		Average		527		529		541		Average		Min.	Max.
Pulse	Resp.	Pulse	Resp.	Pulse	Resp.	Pulse	Resp.	Pulse	Resp.	Pulse	Resp.	Pulse	Resp.	Pulse	Resp.	Pulse	Resp.		
Feb. 23	69	22	70	26	69	28	25	65	25	65	25	62	26	68	24	65	25	58	67
Feb. 26	62	18	69	16	63	18	17	71	16	58	20	58	20	57	16	62	17	40	46
15 grams iodinated casein daily																			
Mar. 5	72	20	80	24	73	21	22	62	17	65	16	63	22	63	22	64	18	42	54
Mar. 11	76	28	80	30	75	28	29	60	15	64	17	62	20	62	20	62	17	45	57
Mar. 20	76	22	72	20	74	25	74	64	18	65	16	64	22	64	22	64	19	48	54
Mar. 26	76	22	84	30	81	33	28	62	22	60	22	64	28	64	28	62	24	54	65
Iodinated casein discontinued																			
Apr. 2	64	18	72	25	71	26	23	72	26	80	33	68	27	73	27	73	29	43	66
Apr. 9	64	24	64	32	67	36	31	80	32	68	25	72	24	73	24	73	27	54	70
Apr. 16	64	18	60	22	65	35	25	72	27†	68	24	68	24	68	25	44	60
Apr. 23	60	28	64	25	64	28	27	80	35	72	36	76	36	76	36	55	71
15 grams iodinated casein daily																			
Apr. 30	64	25	72	30	69	42	32	64	27	64	25	64	25	64	26	43	72
May 7*	80	50	88	60	80	50	53	72	24	64	40	68	40	68	32	60	80

* Cows were on pasture during forenoon.

† Animal died with rabies Data not included in average for group.

It may be observed that there was considerable variation in the pulse rate and respiration rate for the cows in the two groups, nevertheless there was a definite tendency for an increase in pulse rate and respiration with iodinated casein feeding. There was also an increase in respiration with an increase in the barn temperature as might be expected.

DISCUSSION

The results obtained in this experiment show clearly that iodinated casein will stimulate an increase in milk production and the percentage fat in the milk. There is some indication of a slight increase in the percentage solids-not-fat and a considerable decrease in the ascorbic acid content of the milk.

The effect on milk production and the composition of the milk was observed after about 4 to 5 days of iodinated casein feeding. Reineke and Turner (11) observed an increase in milk production after the third day when they were feeding from 50 to 100 grams daily. Cows in the declining phase of lactation fed 15 grams of iodinated casein daily (7.5 grams morning and evening) for a period of four weeks showed an increase of from 5 to 20 per cent in milk production and from 25 to 50 per cent in butterfat production.

The increase in butter fat production was due to both an increase in milk production and an increase in the percentage fat content of the milk. There was considerable carry-over effect from one 4-week period to another in the percentage fat content of the milk. There is some evidence that the cows did not reach the peak for fat percentage of the milk in the 4-week feeding periods. It may be seen that the cows in Group 1 continued to rise in the percentage fat of the milk for the second 4-week iodinated casein feeding period. The butter fat percentage appeared to be influenced more by iodinated casein feeding than milk production.

The solids-not-fat content of the milk of cows fed iodinated casein was only slightly increased which was probably not significant. Herman, Graham and Turner (6) obtained only a slight increase in the solids-not-fat content of the milk when thyroxine was injected subcutaneously or when desiccated thyroid was fed orally.

The feeding of 15 grams of iodinated casein daily was sufficient to cause a decrease of approximately thirty-three per cent in the ascorbic acid content of the milk. This is very similar to results reported earlier by Brown, Van Landingham and Weakley (1) when potassium iodide was fed. When fed at the rate of 5 grams daily for two weeks potassium iodide did not cause an increase in the rate of milk production. What effect, if any, iodinated casein may have on the ascorbic acid content of the blood is yet to be determined. Nevertheless one might expect a reduction in the ascorbic acid content of the blood corresponding to the reduction in the ascorbic acid content

of the milk. Recent investigations have shown that the level of ascorbic acid in the blood plasma is closely associated with fertility in dairy cattle. Results obtained by Philips *et al.* (8, 9) with ascorbacidotherapy on sterile bulls and "hard to settle" cows indicate that ascorbic acid is an important factor in breeding efficiency.

Data obtained on the effect of feeding iodinated casein on respiration and pulse rate are very limited, nevertheless there was a tendency for the rate of respiration to increase. There was also an increase of about 10 beats per minute in pulse rate when iodinated casein was fed. This seems to be in line with observations of Reineke and Turner (11).

Changes in body weight were noted but probably did not affect the animal adversely in the short time covered by this experiment.

SUMMARY

The effect of feeding 15 grams of iodinated casein (Protamone) to milking cows in the declining part of their first lactation has been studied and the following results obtained:

1. Changes in milk production and in the composition of the milk were apparent after about four to five days of iodinated casein feeding.
2. Cows in the declining part of lactation showed an increase of 5 to 20 per cent in milk production and from 25 to 50 per cent in butterfat production.
3. During the first four-weeks' feeding of iodinated casein the fat content of the milk was increased by 0.47 to 0.98 per cent above the fat content of the milk at the beginning of the experiment. When iodinated casein was discontinued for four weeks and then fed for a second four-week period, the fat content was increased by 0.90 to 2.03 per cent above the fat content of the milk at the beginning of the experiment.
4. There was only a slight increase in the solids-not-fat content of the milk.
5. There was a decrease of about thirty-three per cent in ascorbic acid content of the milk.
6. There was also an increase in respiration and pulse rate, and a small decrease in body weight.

ACKNOWLEDGMENT

The authors are indebted to Dr. W. Carson Brown of the Department of Dairy Husbandry and to Dr. V. B. Fish of the Department of Agricultural Chemistry for their assistance with the analytical work in connection with this study. The authors are also indebted to Dr. W. R. Graham, Jr., of the Cerophyl Laboratories, Inc., Kansas City, for supplying the iodinated casein (Protamone) used in these trials.

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PROGRAM
THIRTY-NINTH ANNUAL MEETING
OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION
OHIO STATE UNIVERSITY
COLUMBUS, OHIO
JUNE 20-22, 1944

PROGRAM COMMITTEE

GENERAL:

H. P. DAVIS, *Chairman*—University of Nebraska
DWIGHT ESPE—Iowa State College
O. F. GARRETT—M & R Dietetic Laboratories
E. C. SCHEIDENHELM—Michigan State College
L. H. BURGWARD—Ohio State University

EXTENSION:

E. C. SCHEIDENHELM, *Chairman*—Michigan State College
FLOYD ARNOLD, University of Maryland
GERALD HEEBINK—University of West Virginia
CHARLES L. BLACKMAN—Ohio State University

MANUFACTURING:

O. F. GARRETT, *Chairman*—M & R Dietetic Laboratories
G. C. NORTH—Illinois
B. E. HORRALL—Purdue University

PRODUCTION:

DWIGHT ESPE, *Chairman*—Iowa State College
G. W. SALISBURY—Cornell University
K. L. TURK—University of Maryland

REGISTRATION

CAMPBELL HALL

OHIO STATE UNIVERSITY

Meetings will be held in buildings on the campus of Ohio State University and headquarters will be there.

COMMITTEE MEETINGS

Suitable rooms will be available for all committees and for other groups which may desire to meet. L. H. Burgward will have charge of room assignments.

SCHEDULE OF PROGRAMS

(Eastern War Time)

Date and Time	General	Production Section	Extension Section	Manufacturing Section
<i>Tuesday</i> <i>June 20, 1944</i> 9: 00-12: 00 1: 00-4: 00	Opening Session	Section A Section B	Section	Section A Section B
4: 00- 5: 00 7: 00	Committees Informal Get Together	Committees Symposium	Committees	Committees
<i>Wednesday</i> <i>June 21, 1944</i> 9: 00-11: 00 11: 00-12: 00 1: 00- 4: 00		Section A Section B Joint Business Meeting	Section	Section A Section B Business Meeting
4: 00- 5: 00 7: 00	Post War Problems in Dairying Committees Reception	Committees	Committees	Committees
<i>Thursday</i> <i>June 22, 1944</i> 9: 00-11: 00 11: 00-12: 00 1: 00- 3: 30 3: 30 7: 00		Joint Symposium Business Meeting	Business Meeting	Symposium Business Meeting
	Latin American Dairying Business Meeting Association Banquet			

PHOTOGRAPHIC PROJECTION

Projection equipment will be available if required. It is the hope of the committee, however, that tabular and graphic material will be presented in mimeograph form rather than as slides.

INVITATION TO VISIT OHIO EXPERIMENT STATION

The administration of the Ohio Experiment Station and the members of the Dairy Department staff cordially invite you to visit the Experiment Station at Wooster on your way to or from the Dairy Science meetings. In addition to the regular experimental herd, there is a special herd and farm devoted entirely to pasture research, an experimental forage harvester, and extensive batteries of experimental silos.

Wooster is 90 miles north of Columbus on state routes 3, 5, and 30. It is readily accessible by bus from all directions and by rail from east and west.

GENERAL PROGRAM

*(Eastern War Time)**Tuesday, June 20, 1944*

- 9:00-12:00 OPENING SESSION. Campbell Hall, Room 200.
A. C. DAHLBERG, *President, American Dairy Science Association*, Presiding.
Introduction of Officers and Guests.
Address of Welcome—DR. HOWARD L. BEVIS, *President, The Ohio State University*.
Response and Presidential Address—A. C. DAHLBERG, *President, American Dairy Science Association*.
Guest Speaker.
Announcements.
- 1:00- 4:00 SECTIONAL MEETINGS.
Production Section A, Feeds and Feeding, Room 100, B & Z Bldg.
Production Section B, Physiology and Nutrition, Room 209, B & Z Bldg.
Manufacturing Section A, Bacteriology, Room 302, Campbell Hall.
Manufacturing Section B, Chemistry, Room 200, Campbell Hall.
Extension Section, D.H.I.A. work, Room 206, H & F Bldg.
- 4:00- 5:00 COMMITTEE MEETINGS.
- 7:00- 9:00 Production Section Symposium, Room 100, B & Z Bldg.
Relation of Body Size to Milk Yield.
A. The Energy-Size Basis of Measuring Milk Yield—W. L. GAINES, *University of Illinois*.
B. The Significance of Body Size in Milk Production—SAMUEL BRODY, *University of Missouri*.
C. Pertinent Data on the Problem—MAX KLEIBER, *University of California*.
D. How the Size Problem Affects Breed Associations—C. T. CONKLIN, *Ayrshire Breeders Association*.
- 7:00 INFORMAL GET TOGETHER—*Faculty Club, Ohio State University*.

Wednesday, June 21, 1944

- 9:00-11:00 SECTIONAL MEETINGS.
Production Section A, Feeds and Feeding, Room 100, B & Z Bldg.

Production Section B, Animal Breeding, Room 209, B & Z Bldg.

Manufacturing Section A, Butter and Cheese, Room 203, Campbell Hall.

Manufacturing Section B, Milk and Other Dairy Products, Room 200, Campbell Hall.

Extension Section, Room 206, H & F Bldg.

11:00-12:00 **BUSINESS MEETINGS OF SECTIONS.**

Production and Extension, Joint Business Meeting, Room 206, H & F Bldg.

Manufacturing, Business Meeting, Room 200, Campbell Hall.

1:00- 4:00 **GENERAL SESSION.**

Post War Problems of Dairying, Room 200, Campbell Hall.

A. Why Different Problems After the War—E. L. ANTHONY, *Michigan State College*.

B. Is Dairy Expansion to Continue?—EARL WEAVER, *Michigan State College*.

C. Improving a Post War Testing Program—C. T. CONKLIN, *Ayrshire Breeders' Association*.

D. Post War Demand for Livestock—O. E. REED, *Bureau of Dairy Industry*.

E. Recommended Nutrient Allowances for Dairy Animals—J. K. LOOSLI, *Cornell University*.

F. Nutrition Education as a Safeguard Against Post War Problems—E. M. HARMON, *National Dairy Council*.

G. Problems of Farm Milk Collection Delivery to Plants and Retail Delivery of Milk—LELAND SPENCER, *Cornell University*.

H. Problems Pertaining to Processing, Manufacturing, and Distribution of Dairy Products in the Post War Period—H. H. SOMMER, LELAND SPENCER, A. W. FARRALL AND R. J. RAMSEY. *A Committee Report*.

4:00- 5:00 **COMMITTEE MEETINGS.**

7:00 **RECEPTION, Faculty Club.**

Thursday, June 22, 1944

9:00-11:00 **SECTIONAL MEETINGS.**

Manufacturing Section Symposium, Room 200, Campbell Hall.

Dehydrated Milk and Milk Products.

A. Types of Dehydrated Milk and Milk Products—P. H. TRACY, *University of Illinois*.

- B. Standard Specifications and Methods of Analysis of Dried Milk—LT. ROBERT REMALEY, *Subsistence Research and Development Laboratory, U. S. Army Quartermaster Corps.*
- C. Use of Anti-oxidants in the Manufacture and Storage of Dried Whole Milk—S. T. COULTER, *University of Minnesota.*
- D. Packaging of Dehydrated Milk and Milk Products—MAJOR JAMES D'A. CLARK, *Subsistence Research and Development Laboratory, U. S. Army Quartermaster Corps.*
- E. The Future of the Dehydrated Milk and Milk Products Industry—J. T. WALSH, *American Dry Milk Institute.*

Production and Extension Symposium, Room 206, H & F Bldg.

Mastitis and the Feed Situation.

- A. Mastitis from the Dairyman's Stand Point—T. S. SUTTON, *Ohio State University.*
 - B. Modern Methods of Treating Mastitis—C. S. BRYAN, *Michigan State College.*
- Discussion
- C. The Feed Situation—WALTER BERGER, *Chairman of the Feed and Livestock Division, Food Production Administration.*

Discussion

11:00-12:00 **Sectional Business Meetings**—Production, Room 100, B & Z Bldg.; Manufacturing, Room 200, Campbell Hall; and Extension, Room 206, H & F Bldg.

1:00- 3:30 **General Session, Room 200, Campbell Hall.**

Latin American Dairying.

- A. Dairying and Agriculture—R. E. HODGSON, *Bureau of Dairy Industry.*
- B. Dairy Products in Latin America—A. C. DAHLBERG, *Cornell University.*
- C. Thomas Jefferson, The Farmer—O. E. REED, *Bureau of Dairy Industry.*

3:30 **General Business Meeting, Room 200, Campbell Hall.**

7:00 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

SECTIONAL PROGRAMS

EXTENSION SECTION

Tuesday, June 20

Afternoon Session, Room 206, H & F Bldg.

E. C. SCHEIDENHELM—*Chairman*

1:00–1:15 Opening Business Session.

1:15–4:00 Sectional Program.

A. Panel Discussion on D.H.I.A. work—*Leader, E. H. LOVELAND, University of Vermont.*E1. Agreement Forms and D.H.I.A. Organization—*W. T. CRANDALL, Cornell University.*E2. Short Cuts in Record Keeping—*J. F. KENDRICK, Bureau of Dairy Industry.*E3. A Suggested Form for Permanent and Current Records—*J. F. KENDRICK, Bureau of Dairy Industry.*E4. Securing Supervisors During and Immediately Following the War—*C. R. GEARHART, Pennsylvania State College.*E5. Streamlined Testing for Everybody—*G. W. VERGERONT, University of Wisconsin.*E6. Testing in Areas of Small Herds and Scarce Membership—*R. A. CAVE, South Dakota State College.*B. Report of the Committee—*C. R. GEARHART, Pennsylvania State College.*E7. Wartime 4-H Dairy Club Program—*J. C. NAGEOTTE, Pennsylvania State College.*C. Report of 4-H Club Committee—*H. A. WILLMAN, Cornell University.*

4:00–5:00 Committee Meetings.

Wednesday, June 21

Morning Session, Room 206, H & F Bldg.

E. C. SCHEIDENHELM—*Chairman*

9:00–11:00 Sectional Program.

E8. The Forced Ventilation System of Curing Hay in the Mow—*R. G. CONNELLY—Virginia Polytechnic Institute.*E9. A Complete Sire Proving Program—*C. T. CONKLIN, Ayrshire Breeders Association.*

E10. The Task of Getting Quality Bulls Giving Quality Semen—S. J. BROWNELL, *Cornell University*.

E11. Action Program for Rapid Washing Methods—J. M. JENSEN, *Michigan State College*.

D. Report of Quality and Marketing Committee—E. WALLENFELDT, *University of Wisconsin*.

E. Report of States with Exhibits and Committee on Teaching Ideas—R. T. HARRIS, *University of Wisconsin*.

F. Report of Type Rating Committee—J. W. LINN, *Kansas State College*.

G. Report of Dairy Cattle Health Committee—C. W. REEVES, *University of Tennessee*.

H. Report of Dairy Farm Management Committee—M. J. REGAN, *University of Missouri*.

11:00-12:00 **Joint Business Meeting**—Production and Extension Sections, *Room 206, H & F Bldg.*, E. C. SCHEIDENHELM, *Presiding*.

A. Report of Dairy Cattle Breeding Committee—E. J. PERRY, *Rutgers University*.

B. Report of Breeds Relations Committee—H. A. HERMAN, *University of Missouri*.

C. Report of Pasture and Roughage Committee—R. B. BECKER, *University of Florida*.

Afternoon Session

1:00- 4:00 **General Session**—Post War Problems of Dairying. (See General Program.)

4:00- 5:00 **Committee Meetings.**

Thursday, June 22

Morning Session

9:00-11:00 **Joint Symposium**—Production and Extension Sections, *Room 206, H & F Bldg.*

Mastitis and Feed Situation—DWIGHT ESPE, *Presiding*.

See General Program.

11:00-12:00 **Business Meeting**—Extension Section.

Afternoon Session

1:00- 3:30 **General Session**—Latin American Dairying. (See General Program.)

3:30 **General Business Meeting**, *Room 200, Campbell Hall*.

Evening

7:00

Annual Banquet—Installation of Officers, Presentation of Borden Awards.

PRODUCTION SECTION

Tuesday, June 20

Afternoon Sessions

DWIGHT ESPE—*Chairman*

1:00–4:00 Sectional Programs.

SECTION A—Feeds and Feeding, Room 100, B & Z Bldg.,

G. W. SALISBURY, *Presiding*

- P1. Some Factors Affecting the Nutritive Value of Korean Lespedeza Hay—H. A. HERMAN, E. W. SWANSON, AND A. C. RAGSDALE, *University of Missouri*.
- P2. The Feeding Value of Cheat for Dairy Cows—M. H. BERRY, K. L. TURK, AND L. A. MOORE, *University of Maryland*.
- P3. Silage Fermentation Losses—A. E. PERKINS, *Ohio Agricultural Experiment Station*.
- P4. Silage Versus Winter Pasture for Dairy Cows—C. E. WYLIE, B. P. HAZELWOOD, AND L. R. NEEL, *University of Tennessee*.
- P5. Effect of Feeding Corn and Alfalfa Silage on the Fat- and Water-Soluble Vitamins—J. J. STEFANIAK, I. W. RUPEL, AND W. H. PETERSON, *University of Wisconsin*.
- P6. New Feeding Standard for Milk Production—T. A. BAKER, *University of Delaware*.
- P7. Limited Grain Ration Versus All Roughage Ration for Dairy Cattle—F. B. WOLBERG, A. A. SPIELMAN, V. L. MILLER, AND U. S. ASHWORTH, *State College of Washington*.
- P8. Limited Grain Feeding of Dairy Cows—C. E. WYLIE, S. A. HINTON, AND B. P. HAZELWOOD, *University of Tennessee*.
- P9. Carotene Losses in Freshly Cut Plant Tissues—R. K. WAUGH, S. M. HAUGE, AND J. H. HILTON, *Purdue University*.
- P10. Vitamin A Requirements of Dairy Cattle for Normal Growth and Reproduction—J. H. HILTON, J. W. WILBUR, AND S. M. HAUGE, *Purdue University*.
- P11. Carotene Levels for Growth and Reproduction in Dairy Bulls—I. R. JONES, *Oregon State College*.

- P12. Changes in Blood-plasma Carotene Associated with Parturition and Lactation of Jersey Cows—A. H. KUHLMAN AND W. D. GALLUP, *Oklahoma A & M College*.
- P13. Vitamin E in the Nutrition of Cattle—T. W. GULLICKSON, L. S. PALMER, W. L. BOYD, AND F. C. OLSON, *University of Minnesota*.

Paper to be Read by Title

- P13a. The Value of Fat in Alfalfa Hay Rations—I. R. JONES, *Oregon State College*.

SECTION B—Physiology and Nutrition, Room 209, B & Z Bldg., DWIGHT ESPE, *Presiding*

- P14. Studies on Ketosis in Dairy Cattle—J. C. SHAW, *University of Connecticut*.
- P15. Symptoms of Scurvy Observed in a Herd of Dairy Cattle—C. W. DUNCAN, C. F. HUFFMAN, R. MITCHELL, JR., AND J. T. REID, *Michigan State College*.
- P16. The Relation of Vacuum to Speed in Mechanical Milking—VEARL SMITH AND W. E. PETERSEN, *University of Minnesota*.
- P17. Studies of Mammary Gland Carbohydrate Metabolism in Vitro—C. B. KNOTT AND W. E. PETERSEN, *University of Minnesota*.
- P18. Pre-partum Milking—E. A. KEYES, J. J. REID, A. A. BORLAND, A. L. BEAM, AND P. S. WILLIAMS, *Pennsylvania State College*.
- P19. The Use of Simplified Diets in the Study of the Fat Metabolism of the Mammary Gland—O. W. KAUFMANN, *University of Connecticut*.
- P20. The Effect of Two Different Methods of Feeding Cod Liver Oil on Fat Test in Milk—L. A. MOORE, G. T. HOFFMAN, AND M. H. BERRY, *University of Maryland*.
- P21. Further Observations on the Initiation and Maintenance of Lactation in Dairy Cattle—RALPH P. REECE, *New Jersey Agricultural Experiment Station*.
- P22. The Response of Louisiana Milk Cows to Iodinated Casein Feeding—D. M. SEATH, CECIL BRANTON, AND A. H. GROTH, *Louisiana State University*.
- P23. Studies in the Utilization of Thyroprotein by Ruminants—C. W. TURNER AND E. P. REINEKE, *University of Missouri*.

- P24. Studies of Thyroid Physiology by Use of Thiourea and Its Derivatives—E. P. REINEKE, A. B. SCHULTZE, AND C. W. TURNER, *University of Missouri*.
- P25. The Effect of Pitocin on Milk Lipase—PHILIP L. KELLY, *University of Arkansas*.
- P26. Factors Affecting the Chylomicron Count—DWIGHT ESPE AND C. Y. CANNON, *Iowa State College*.

Paper to be Read by Title

- P27. Morphology of the Teat in Relation to Milking and Trauma—W. E. PETERSEN, C. B. KNOTT, AND W. L. BOYD, *University of Minnesota*.

4:00–5:00 Committee Meetings.

7:00 Symposium—Relation of Body Size to Milk Yield. (See General Program.)

Wednesday, June 21

Morning Session

9:00–11:00 Sectional Programs.

SECTION A—Feeds and Feeding—Calf Rearing—Harvesting Roughages, *Room 100, B & Z Bldg.*, DWIGHT ESPE, *Presiding*

- P28. Threshed Peanut Hay as a Roughage for Dairy Cows—A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A & M College*.
- P29. The Use of Urea in Commercial Dairy Feeds—W. H. HASTINGS, *Nutritional Director, Lindsey-Robinson & Company*.
- P30. Corn Silage Made with the Addition of Urea and Its Feeding Value—T. E. WOODWARD, *Bureau of Dairy Industry*.
- P31. Urea Sorghum Silage—GEORGE K. DAVIS, C. L. COMAR, R. B. BECKER, AND P. T. DIX ARNOLD, *University of Florida*.
- P32. Urea Treated Corn Silage Versus Untreated Corn Silage as a Dairy Feed—G. H. WISE, J. H. MITCHELL, J. P. LAMASTER, AND D. B. RODERICK, *Clemson Agricultural College*.
- P33. The Minimum Protein Requirement of Young Holstein Calves—L. E. HARRIS AND J. K. LOOSLI, *Cornell University*.
- P34. Studies on Carotene Conversion in the Young Calf—NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, *University of Wisconsin*.

- P35. Observations on the Pathology of Dairy Calves on Low Vitamin A Diets—E. A. KEYES, S. I. BECHDEL, W. T. S. THORP, AND N. B. GUERRANT, *Pennsylvania State College*.
- P36. The Effect of Sulfa Drugs and Immunity Development Upon Blood Plasma Vitamins A and C in the Young Bovine—NORMAN S. LUNDQUIST AND PAUL H. PHILLIPS, *University of Wisconsin*.
- P37. The Use of Stored Colostrum to Replace Marketable Milk for Calf Feeding—N. N. ALLEN, *University of Vermont*.
- P38. Experiences with the Forage Crop Harvester in Making Grass Silage—C. F. MONROE, *Ohio Agricultural Experiment Station*.
- P39. Development of the Barn Hay-drying System—C. E. WYLIE AND JOHN A. SCHALLER, *University of Tennessee*.

SECTION B—Animal Breeding and Reproduction, Room 209, B & Z Bldg., G. W. SALISBURY, *Presiding*

- P40. Congenital Muscular Contracture and Ankylosis in Dairy Cattle—A. A. SPIELMAN, O. J. HILL, AND E. C. McCULLOCK, *State College of Washington*.
- P41. The Optimum Emphasis on Dam's Records when Proving Dairy Sires—JAY L. LUSH, *Iowa State College*.
- P42. Tendency Toward Mastitis in Two Cow Families—J. M. MURPHY AND K. O. PFAU, *New Jersey Agricultural Experiment Station*.
- P43. The Effect of the Oral Administration of Chloretone to Bulls on the Fertility of the Semen Produced—IRVINE ELLIOTT, *Cornell University*.
- P44. Storage of Bovine Spermatozoa in Diluents Containing Certain Amino Acids—C. E. KNOOP, *Ohio Agricultural Experiment Station*.
- P45. Recent Observations on the Preservation of Bull Semen—HENRY A. LARDY AND PAUL H. PHILLIPS, *University of Wisconsin*.
- P46. Further Studies of the Effect of Dilution Rate on the Fertility of Bull Semen Used for Artificial Insemination—G. W. SALISBURY, IRVINE ELLIOTT, AND N. L. VANDEMARK, *Cornell University*.
- P47. Conception Rate in Dairy Cattle by Artificial Insemination at Various Intervals Before and After

Ovulation—G. W. TRIMBERGER AND H. P. DAVIS, *University of Nebraska*.

P48. The Methylene Blue Reduction Test and Its Relation to Other Measures of Quality in Bull Semen—G. W. SALISBURY, ERNEST MERCIER, AND N. L. VANDEMARK, *Cornell University*.

P49. The Influence of Pregnancy on the Body Weight of Dairy Cows—D. N. PUTNAM AND H. O. HENDERSON, *University of West Virginia*.

P50. Factors to Consider in Long Distance Semen Shipping—H. A. HERMAN AND E. W. SWANSON, *University of Missouri*.

11:00-12:00 **Joint Business Meeting**—Production and Extension Sections, *Room 206, H & F Bldg.*, E. C. SCHEIDENHELM, *Presiding*.

A. Report of Dairy Cattle Breeding Committee—E. J. PERRY, *Rutgers University*.

B. Report of Breeds Relations Committee—H. A. HERMAN, *University of Missouri*.

C. Report of Pasture and Roughage Committee—R. B. BECKER, *University of Florida*.

Afternoon Session

1:00- 4:00 **General Session**—Post War Problems of Dairying. (See General Program.)

4:00- 5:00 **Committee Meetings.**

Thursday, June 22

Morning Session

9:00-11:00 **Joint Symposium**—Production and Extension Sections, *Room 206, H & F Bldg.*

Mastitis and Feed Situation—*See General Program.*

11:00-12:00 **Business Meeting**—Production Section, *Room 100, B & Z Bldg.*

Afternoon Session

1:00- 3:30 **General Session**—Latin American Dairying, *Room 200, Campbell Hall.*

3:30 **General Business Meeting**, *Room 200, Campbell Hall.*

Evening

7:00 **Annual Banquet**—Installation of Officers and Presentation of Borden Awards.

MANUFACTURING SECTION

Tuesday, June 20

Afternoon Session

P. F. SHARP—*Chairman*

1:00- 4:00 Sectional Programs.

SECTION A—Bacteriology, *Room 302, Campbell Hall*

- M1. Factors Which Influence the Apparent Survival of Heat-Treated Bacteria—F. E. NELSON, *Iowa State College*.
- M2. A Correlation of the Resazurin Test Read with an All-Purpose Lovibond Comparator at 10, 30, and 60 Minutes with the Standard Plate Count of Milk—S. IRENE JORGENSEN AND N. S. GOLDING, *State College of Washington*.
- M3. Mastitis and the Plate Count of Milk—E. O. ANDERSON, *University of Connecticut*.
- M4. Behavior of Bacteria in Certain Gases under Pressure—M. J. PRUCHA, *University of Illinois*.
- M5. Bactericidal Property of Some Acids, Wetting Compounds, and Acid Cleaners—M. J. PRUCHA, *University of Illinois*.
- M6. Farm Sources of *Oospora Lactis*—E. R. GARRISON, *University of Missouri*.
- M7. Further Observations on the Use of the Propionates as Inhibitors of Mold on the Surface of Butter—J. C. OLSON AND H. MACY, *University of Minnesota*.
- M8. The Mold Mycelia Count as an Index of Quality of Butter—LT. P. R. ELLIKER AND B. E. HORRALL, *Purdue University*.
- M9. An Aerobacter Species in Whey Cream as a Cause of a Medicinal Flavor Encountered in Butter—T. J. CLAYDEN, *University of Arkansas*.

SECTION B—Chemistry, *Room 200, Campbell Hall*

- M10. Some Chemical and Physical Properties of Washing Powder—P. S. LUCAS, *Michigan State College*.
- M11. Factors Entering into the Testing of Some Cream for Extraneous Matter—K. M. RENNER, *Texas Technological College*.
- M12. The Problem of Extraneous Matter in Cheddar Cheese—W. V. PRICE AND RAYMOND MIERSCH, *University of Wisconsin*.

- M13. Correlating Lactic Acid Determination with Practical Milk Quality Test in Grading Milk for Manufactured Dairy Products—L. A. GOULD, *Michigan State College*.
- M14. Use of Conductivity Measurements for Detecting Neutralization of Sweet Cream Buttermilk—S. T. COULTER AND R. W. KUNKEL, *University of Minnesota*.
- M15. An Improved Technique for the Determination of the Volatile Acidity of Cheese—K. L. SMILEY AND A. C. DAILBERG, *Cornell University*.
- M16. A Study of Some of the Substances Adsorbed on the Fat Globules of Milk—ROBERT JENNESS, *University of Minnesota*.
- M17. Some Factors Affecting the Inversion of Sucrose—T. R. FREEMAN AND E. L. FOUTS, *University of Florida*.
- M18. Study of the Peroxide Value of Stored Spray-Dried Whole Milk Powder—HARRY PYENSON, P. H. TRACY, AND J. E. TRIMBLE, *University of Illinois*.
- M19. The New Method for the Determination of Lipase in Milk. PHILIP L. KELLY, *University of Arkansas*.

4:00–5:00 Committee Meetings.

Wednesday, June 21

Morning Session

P. F. SHARP—*Chairman*

9:00–11:00 Sectional Programs.

SECTION A—Butter and Cheese, Room 203,
Campbell Hall

- M20. The Influence of Butter Cultures and of Butter Flavors on the Quality of Butter—H. C. OLSON AND P. E. JOHNSON, *Oklahoma A and M College*.
- M21. Effect of Various Bacteria on Diacetyl Content and Flavor of Butter—LT. P. R. ELLIKER, *Purdue University*.
- M22. The Effect of Storage Temperatures on the Keeping Quality of Butter—C. W. STARK, J. R. CAMPBELL, AND E. S. GUTHRIE, *Cornell University*.
- M23. Butter Studies—E. S. GUTHRIE, *Cornell University*.
- M24. Action of Lipases from Various Sources on the Fat of Cheddar Cheese—F. J. BABEL, *Iowa State College*.

- M25. Combining Lactic and Bulgaricus Fermentation in Cheese Making to Prevent Gas Formation in Cheese Ripening—N. S. GOLDING, *State College of Washington*.
- M26. Does the Growth of Blue Mold in Roquefort Type Cheese Change Its Bacterial Flora?—N. S. GOLDING, *State College of Washington*.
- M27. Effect of Heat Treatments of Milk on Quality and Ripening of Cheddar Cheese—A. O. CALL AND W. V. PRICE, *University of Wisconsin*.
- M28. Relation of Corn and Alfalfa Silage to the Quality of Cheese and Its Carotene and Vitamin A Content—W. V. PRICE, K. HIGUCKI, AND W. H. PETERSON, *University of Wisconsin*.

SECTION B—Milk and Other Dairy Products,
Room 200, Campbell Hall

- M29. Two Years' Experience in Deaerating Milk—E. S. GUTHRIE, *Cornell University*.
- M30. The Relationship of the Individuality of the Cow to the Production of Rancid Milk—W. A. KRIENKE, *Oklahoma A & M College*.
- M31. Milk as a Frozen Food—J. G. LEEDER AND F. J. DOAN, *Pennsylvania State College*.
- M32. The Utilization of Skimmilk in Ice Cream Mix—W. S. ARBUCKLE, C. N. SHEPARDSON, AND H. M. WALLING, *A & M College of Texas*.
- M33. Factors Affecting the Oxygen Content of the Gaseous Phase of Packaged Whole Milk Powder—JOHN HETRICK AND P. H. TRACY, *University of Illinois*.
- M34. The Keeping Quality of Commercial Dried Whole Milks Packaged in Air and in Nitrogen—G. R. GREENBANK, P. A. WRIGHT, AND E. F. DEYSHER, *Bureau of Dairy Industry, U.S.D.A.*
- M35. Further Observations Dealing with the Behavior of Ascorbic Acid in Evaporated Milk—D. V. JOSEPHSON AND F. J. DOAN, *Pennsylvania State College*.
- M36. A Comparison of Different Types of Sweetening Agents in the Preservation of Condensed Milk—W. A. HOSKISSON AND P. H. TRACY, *University of Illinois*.
- M37. Iron Content of Evaporated Milk in Relation to Greenish-Dark Discoloration in Mixtures of Coffee

and Evaporated Milk—W. C. COLE AND N. P. TARASSUK, *University of California*.

M38. Can We Hold Our Wartime Marketing Gains in Post-War Adjustments—C. G. McBRIDE, *Ohio State University*.

11:00-12:00 **Business Meeting**—Manufacturing Section, *Room 200, Campbell Hall*.

Afternoon Session

1:00- 4:00 **General Session**—Post War Problems of Dairying. (See General Program.)

4:00- 5:00 **Committee Meetings**.

Thursday, June 22

Morning Session

P. F. SHARP—*Chairman*

9:00-11:00 **Symposium**—Dehydrated Milk and Milk Products, *Room 200, Campbell Hall*.

See General Program.

11:00-12:00 **Business Meeting**—Manufacturing Section, *Room 200, Campbell Hall*.

Afternoon Session

1:00- 3:30 **General Session**—Latin American Dairying. (See General Program.)

3:30 **General Business Meeting**, *Room 200, Campbell Hall*.

Evening

7:00 **Annual Banquet**—Installation of Officers and Presentation of Borden Awards.

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RED MOLD ON BLUE CHEESE¹

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During recent years there has been a great increase in the blue cheese production of the United States, particularly in the Middle West. As new plants have come into production and the older plants have increased their output, various problems have become important. One of these is the appearance of red areas of mold growth on the surfaces of cheese in certain curing rooms.

Commonly the red mold is first evident on the cheese as small, bright-red colonies which may be few or very numerous. The colonies usually are noted in 10 days to several weeks and increase in size, often rather rapidly, until they are 1 inch or more in diameter. If the colonies are rather numerous they grow together and form large red areas which make up a considerable portion of the cheese surface. Both the colonies and areas of mold growth have more or less irregular edges. In some instances blue or green mold growth is evident in the red growth, especially when the latter has been present for some time, but ordinarily the red growth continues to be dominant in the areas in which it has developed. The red colonies and areas are very conspicuous on cheese because of their bright-red color which contrasts sharply with the colors of the other growth on the surfaces of the cheese.

ISOLATION OF THE RED MOLD

Although the general appearance of the red colonies suggests that isolation of the mold would be simple, difficulties were encountered in the early attempts. Material taken from the colonies and smeared on various media usually gave such a heavy growth of bacteria, yeasts and *Penicillium roqueforti* that colonies of the red mold were not obtained. Eventually a cheese agar which had been developed for the isolation of *Bacterium linens* (1) was found to be fairly satisfactory.

To prepare the medium, 100 g. of ripened cheese is suspended in 300 ml. of distilled water containing 10 g. of potassium citrate. When the cheese is well distributed the mixture is warmed to about 50° C. and placed in a

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cylinder for separation of the fat. Thirty minutes are enough to give reasonably complete separation. Only the aqueous portion of the suspension is used in the agar so the fat is removed by suction. Ten g. of peptone, 50 g. of sodium chloride, 2 g. of sodium oxalate and 15 g. of agar are dissolved in 700 ml. of distilled water. The cheese suspension is added to this mixture and the reaction is adjusted to pH 7.4. The agar is dispensed in bottles and sterilized in the autoclave for 25 minutes at 15 pounds pressure. When plates are to be poured, the melted and cooled agar must be thoroughly agitated to distribute the suspended cheese solids.

Material taken from the red colonies on cheese and smeared on the surface of the cheese agar regularly showed growth of various organisms but usually some areas were found on which the red mold was growing away from other species. By picking material from such areas and smearing on another cheese agar plate and repeating this procedure, pure cultures eventually were obtained, although a considerable period was required. Incubation of the plates at 18° to 21° C. with a relatively high humidity was satisfactory.

IDENTITY OF THE RED MOLD

Laboratory examination of the red mold disclosed it to be a fungus which has been known as a contaminant of cheese for many years. It grows very

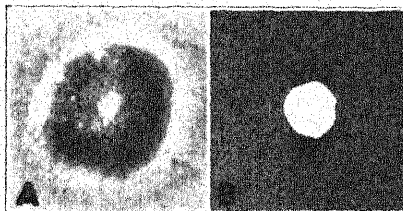


FIG. 1. Growth of *Sporendonema casei* on cheese agar (A) and potato dextrose agar (B) at 20° C., after 10 days.

slowly on the ordinary media used in mycological work, such as potato dextrose or Czapek's solution agar, but much more rapidly on the cheese medium mentioned above. This relationship explains the difficulty encountered when isolations were first attempted. On the cheese medium, the mycelium was floccose, white at first but soon becoming orange-red as the fruiting developed. On potato dextrose agar the growth was much slower, as is shown in figure 1 which illustrates colonies of the same age on the two media. On potato dextrose agar a further difference was noted; the red color was suppressed in the fungus but diffused into the medium turning it dark red. Optimum growth occurred between 15° and 20° C.

Sporulation was initiated by the production of erect fruiting hyphae on the surface of a colony. These hyphae were club-shaped and sparingly

branched, with septa at irregular intervals. They were two to three times the diameter of the mycelial threads that bore them (figure 2). Spores were laid down in the cells of the sporophores by the formation of thick walls within the walls of the sporophores. They were filled with oil drops which gave the characteristic color to the colonies. The spores were disc-shaped with rounded corners and were liberated by the breaking of the hyphal wall which surrounded them. They were variable in size depending on their position in the club-shaped fruiting hyphae; their longest diameter varied from 6 to 9 microns. These spores, being produced within the walls of the hyphae which bear them and also because of the thick wall, would be best denoted as chlamydospores, following the proposal of Vuillemin (17),² rather than conidia so that they would not be confused with similar thin-walled spores (arthrospores) produced on the hyphae of such fungi as

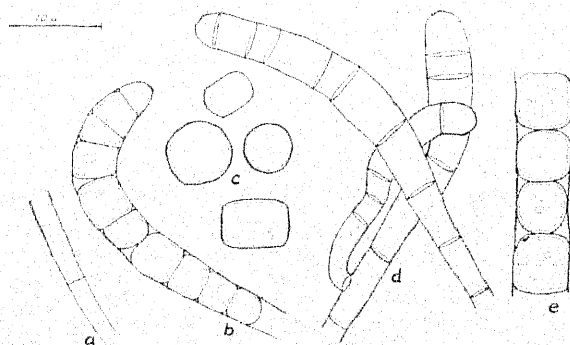


FIG. 2. Chlamydospores and sporophores of *Sporendonema casei*. a. Mycelium. b. Mature sporophore showing method of fracture. c. Mature conidia. d. Immature sporophores showing method of branching. e. Fragment of sporophore containing four mature chlamydospores.

Geotrichum candidum (*Oospora lactis*) as described by Langeron and Talice (8).

Taxonomically the name that should be used for the red mold is very difficult to determine. In 1826, Desmazières (6) described a red mold on cheese under the name of *Sporendonema casei* which he pointed out had been earlier denoted *Mucor crustaceus* Bulliard, *Aegerita crustacea* DeCandolle, *Oidium rubens* Link, and *Scpedonium caseorum* Link, but which did not fit into any of these genera because of the endogenous production of the spores, a character which had not been observed by earlier workers. Corda (5), in

² In 1910, Vuillemin (17) divided the asexual spore forms found in the Fungi Imperfecti into two main groups (a) *conidia vera*, i.e., spores abstricted from the tip of a conidiophore and (b) *thallospores* which are part of the hypha bearing them. The thallospores were in turn divided into three types (a) *arthrospores* which arise by fragmentation of the hypha, (b) *blastospores* which arise as globular buds and (c) *chlamydospores*, thick-walled spores, laid down either terminally or intercalarily.

1838, denied the internal origin of the spores and transferred the fungus to his genus *Torula* and Saccardo (14), in 1880, emended the genus *Oospora* and, in 1882, included the red mold in it under the name *Oospora crustacea* (Bull.) Sacc. Since then this name has often been used for the organism. However, in 1924, Loubière (11) in his study of the molds found on cheese returned to *Sporendonema casei* as a designation for the red fungus. In the meantime the fungus and its name had been variously interpreted because of the lack of uniformity of usage among workers in this group of fungi. Berkhout (2), in her discussion of the molds in the group, treated it in a literature review under the name *Oospora crustacea*. Sumstine (15) stated: "The genus *Sporendonema* was first described by Desmazières with one species, *S. casei*. This is generally considered congeneric with the so-called *Oospora lactis* (Fres.) Sacc. and synonymous with *Oospora crustacea* (Bull.) Sacc." Unfortunately *Sporendonema casei* was not available to Sumstine for, had it been, he doubtless would have recognized the characters which remove it from *Oospora* or *Oosporoidea*, which latter genus he erected to receive *Oospora lactis*, and treated it in its proper place. Biourge (3) discussed the species in his monograph on the genus *Penicillium* among the species of the section *Anomala* but did not make a change of name. Thom (16) said of this culture: "There is no suggestion of relationship to *Penicillium* in this culture." Still more recently Linder (10), in his revision of the genus *Oidium*, suggested that his proposed usage of the name *Oidium* might clear the way "for accepting *Sporendonema* of Desmazière for those forms represented by *Oidium* or *Oospora lactis* and *casei*," thus continuing to include *O. lactis* in the same genus with the cheese mold. In the meantime the name *Sporendonema* had been applied by Oudemans (12), in 1886, to another species, *S. terrestre*, by an emendation of Desmazières' description, a change accepted by Saccardo (14) and Lindau (9). Still later, in 1934, Ciferri and Redaelli (4) and Redaelli and Ciferri (13) again emended the genus to include certain fungi that were human pathogens. Dodge (7, p. 198) stated of Oudemans' fungus: "It does not seem related to Desmazières' original species" and that Ciferri and Redaelli "have used the name for wholly unrelated organisms."

The question of the name for the red cheese mold, then, becomes a question of the propriety of using *Sporendonema* or *Oospora* for this fungus, as well as one of the specific name. *Oospora* as a genus seems questionable (8) and certainly has not been considered to have endogenous spores, such as are found in the cheese mold. Further, the spores in that fungus are thick-walled chlamydospores rather than the thin-walled arthrospores characteristic of *O. lactis*, a species that has been suggested as congeneric. Hence Desmazières' name *Sporendonema* would seem to be the name to retain together with his specific name *casei* which was recognized by Fries. Such a procedure would mean a return to the Desmazières' genus and a discarding

of the emendations thereof, and would clearly separate *Sporendonema casei* from *Oospora lactis*, a fungus that is not congeneric.

CONTROL OF THE RED MOLD

In some curing rooms in which the red mold is very conspicuous on the cheese surfaces, it appears to be difficult to control; at least, the plant operators believe this is the case and certainly the mold quickly develops on young cheese going into the rooms. In other curing rooms in which the red mold has been noted, but in which it has never been conspicuous, the mold appears to have been controlled by adequate cleaning of the cheese surfaces through scraping or washing. A factor that probably is of importance in this general connection is the period the cheese is in a curing room; with short curing there apparently is less chance for the mold to become thoroughly established in a room than with long curing.

A number of cheese were dipped in, or painted with, heated petrolatum to which calcium propionate and propionic acid had been added, the cheese first being dried as completely as possible by exposing them at room temperature to an electric fan. The mold inhibitors were used in different amounts and the petrolatum, which was said to melt at 55° to 58° C., was heated to 71°, 88° or 93° C. Considerable difficulty was encountered in getting a thin and uniform layer of petrolatum on the cheese and even at curing room temperatures the petrolatum remained soft and slipped when the cheese were handled.

The petrolatum tended to limit the surface growth on the cheese and most of the conspicuous growth was very near the punch holes which were made after the treatment with petrolatum. In general, it appeared that the petrolatum offered some interesting possibilities in preventing growth of the red mold and other organisms on cheese. However, a type of material which would be firmer than the petrolatum at curing room temperatures probably would be better.

SUMMARY

The red mold which develops on the surface of blue cheese in certain curing rooms was most easily isolated on a special cheese agar. Apparently the logical designation for the mold is *Sporendonema casei*.

Covering blue cheese with petrolatum containing mold inhibitors offers some interesting possibilities in preventing growth of the red mold on the cheese when the normal cleaning of the cheese does not.

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OBSERVATIONS ON THE USE OF ROLLER PROCESS SWEET CREAM BUTTERMILK POWDER IN ICE CREAM*

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*Division of Dairy Husbandry, University of Minnesota,
University Farm, St. Paul, Minn.*

INTRODUCTION

In recent years, the ice cream industry has used limited quantities of condensed and powdered sweet cream buttermilk as sources of milk solids-not-fat for ice cream. Whitaker (12), in 1936, reviewed the status of the use of sweet cream buttermilk in ice cream and considered the interest in this product as not being very great at that time. He noted that the ice cream manufacturer became interested in the use of the product when the price of skimmilk solids was high, or, in some localities, where the supply of sweet cream buttermilk was large. The demand for milk solids has been increasing at such a rapid pace that today the by-products of the dairy industry are being utilized as sources of human food to a much greater extent than ever before. In past years, the greater bulk of creamery buttermilk has been utilized as animal feed. Attempts are being made, however, to convert more of this by-product into channels of human consumption, and there has been a marked increase in the supply of this product in response to the sharp rise in demand for all forms of milk solids-not-fat. In those localities where concentrated sweet cream buttermilk products are available, many ice cream manufacturers are turning to these products as supplementary sources of milk solids.

REVIEW OF LITERATURE

Combs (3) was the first to report on the use of powdered sweet cream buttermilk in ice cream. From the results he obtained, using consumer preference tests, it appeared that ice cream which contained sweet cream buttermilk powder was considered equal, or slightly superior, to that which contained skimmilk powder as the source of milk solids-not-fat.

The use of sweet cream buttermilk has been found by some investigators to improve the whipping ability of ice cream mixes containing butter as the source of butterfat. Other investigators have reported less favorable results. Caulfield and Martin (2) concluded from their studies that there was no practical value in using powdered buttermilk as a means of improving the whipping ability of ice cream mixes; however, they observed a slight

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improvement. Tracy and Ruehe (9) concluded that no improvement in whipping ability resulted when buttermilk was used with butter in ice cream mixes. Whitaker (11) reported a saving of four minutes in the time required to reach the desired overrun when using buttermilk in a butter mix. The maximum overrun obtainable was also markedly increased over that of a butter and skimmilk control. He also noted that the buttermilk mixes showed less fat clumping than the control, and this phenomenon was accompanied with a lower basic viscosity. Whitaker believed that his findings suggested the presence of a material capable of stabilizing the fat emulsion in the mixes containing buttermilk and stated that the material may be lecithin. Walts and Dahle (10) conducted an experiment which paralleled that of Whitaker. They found an average saving of two minutes in whipping time when buttermilk was used along with butter in ice cream mixes, as compared with similar mixes made with butter and skimmilk. They believed that the failure of butter mixes to whip as readily as cream mixes may be explained as due to the lack of the lecithin-protein complex and that the presence of this complex in relatively large amounts in the buttermilk may have been responsible for the results they obtained. Whitaker (12), in a discussion of the advantages of using buttermilk in ice cream called attention to its higher butterfat content in comparison with skimmilk, its superior emulsifying properties, and its ability to impart a rich creamy flavor.

EXPERIMENTAL METHODS

The data included in this report were obtained from studies dealing with the use of roller process sweet cream buttermilk powder as the additional source of milk solids-not-fat for ice cream. The experiments were conducted on a comparative basis and roller process skimmilk powder was used as the additional source of milk solids-not-fat for all control ice creams.

Method of obtaining the buttermilk and skimmilk products. Arrangements were made with a nearby Twin City plant to secure skimmilk and raw sweet cream which had been obtained from the separation of a single vat of mixed whole milk of high quality. The skimmilk was flash pasteurized at about 165° F. prior to its shipment. The cream was standardized to the desired butterfat content with a portion of the skimmilk from the same separation and was then pasteurized at 165° F. for 30 minutes, cooled to 40° F., and held overnight at this temperature before churning. A standard churning procedure was employed, and the buttermilk was recovered as soon as the butter granules were of proper size.

The skimmilk and sweet cream buttermilk were dried on a "Bufflovak" atmospheric drum drier at the University of Minnesota creamery. In each trial, the fluid products were preheated to 155° F. before drying and were not precondensed. The operation of the drier was standardized before collecting the powder samples by first drying at least 200 pounds of the

fluid skimmilk. After collecting the samples of powdered skimmilk, the buttermilk was dried immediately without interrupting the operation of the drier in order that both products could be obtained under similar operating conditions. All powders were analyzed for moisture, butterfat, and titratable acidity before use in ice cream mixes.

Method of processing and freezing the ice cream mixes. Eighty-five-pound mixes were prepared which had the following composition: 12.0 per cent butterfat, 11.0 per cent milk solids-not-fat, 15.0 per cent cane sugar, 0.30 to 0.35 per cent 225 Bloom gelatin, and 38.30 to 38.35 per cent total solids. All mixes were pasteurized at 160° F. for 30 minutes. The mixes were homogenized at the pasteurization temperature in a two-stage Colony homogenizer at pressures of 2,500 pounds and 500 pounds on the first and second stages, respectively. They were conducted directly from the homogenizer to a direct-expansion surface cooler and cooled to between 36° and 40° F. They were then aged at a temperature of 40° F. for a period of from 18 to 24 hours before freezing.

All mixes were frozen in a Creamery Package direct-expansion, batch freezer which had a capacity of 40 quarts. The batches were weighed accurately before being placed in the freezer and equal portions were prepared for freezing. Pure vanilla extract was added at the rate of 4 ounces per 40-pound batch.

Extreme care was taken to secure uniform freezing conditions when freezing a series of mixes in order that comparative freezing and whipping data could be obtained. A preliminary batch of mix was always frozen to chill the freezer and to standardize operating conditions. The time of freezing was standardized and was held constant for each series of mixes. All samples of ice cream used for meltdown tests and scoring were taken at an overrun of 90 per cent and placed immediately in a hardening room maintained at about -10° F.

Methods of testing the ice mixes and ice cream. The total solids content was determined on all ice cream mixes according to the method outlined by Mojonnier and Troy (5).

The titratable acidity was determined on duplicate samples of each ice cream mix within 18 hours after processing. A 9-gram sample of undiluted mix was titrated with N/10 sodium hydroxide using phenolphthalein as the indicator. The acidity was calculated as per cent lactic acid.

The pH of the mixes was determined with a Coleman Model 3 Electrometer.

The original, apparent, and basic viscosities were determined on all mixes. The original viscosity was determined on a sample of the fresh mix immediately after cooling. The apparent and basic viscosities were those of the aged mix after an aging period of about 18 hours. A sample of unagitated mix was used for the apparent viscosity measurements, whereas

the basic viscosity was that of a sample of the aged mix after it had been passed through a hand emulsifier operated at the rate of 78 strokes per minute, according to the method of Penczek and Dahlberg (6). The viscosities were determined with an Improved MacMichael viscosimeter operated at 20 revolutions per minute and using a standard disc plunger suspended by a number 31 wire. The wire was calibrated with a 60 per cent sucrose solution and all readings were converted to viscosity in centipoises. All viscosity readings were taken at a temperature of 40° F., and the operations were conducted in a room maintained at about 40° F.

All overrun determinations were made at the freezer by the use of a Mojonnier Overrun Tester. The tester was accurately calibrated for each batch of mix immediately before freezing.

A general indication of whipping ability was secured for all ice cream mixes by checking the time required to whip to an overrun of 90 per cent. In each case duplicate batches were frozen for an extended whipping ability test. The frozen mix was allowed to whip until the maximum overrun had been reached and the overrun and temperature of the ice cream were determined at one-minute intervals throughout the whipping process. Temperatures were determined with a sub-zero centigrade thermometer graduated in 0.1-degree divisions. The temperature in each case was estimated to the nearest 0.05 degree and all centigrade readings were converted to degrees Fahrenheit.

During one trial, Draw-Rite readings were recorded at minute intervals during the freezing and whipping of each mix and coincidentally with each overrun determination. It was believed that since Draw-Rite readings serve as an indication of the consistency of the ice cream in the freezer, it would be possible to utilize these readings in comparing the consistencies of the ice cream throughout the freezing process.

The melting properties of the ice cream were determined by allowing a pint sample of ice cream to melt at room temperature. The ice cream (a weighed sample) was placed on a wire screen which had been placed over a glass funnel, and the drippings were collected in a beaker. The weight of drainage was determined at regular intervals until melting was nearly complete.

The ice creams were scored for flavor and body and texture by two experienced judges of dairy products. The age of the sample at each scoring was recorded along with the scores and criticisms.

RESULTS

Representative data from three experiments are included in this report. For purposes of discussion these experiments will be referred to as trials A, B, and C.

Trial A. For trial A, a series of four mixes was designed as follows:

Mix number	Source of butterfat	Source of additional milk solids-not-fat
1 (control)	cream	R.P.* skimmilk powder
2	cream	R.P. sweet cream buttermilk powder
3 (control)	unsalted butter	R.P. skimmilk powder
4	unsalted butter	R.P. sweet cream buttermilk powder

*Roller process.

The skimmilk and buttermilk powders were derived from the same lot of whole milk and supplied in each case about 46 per cent of the milk solids-

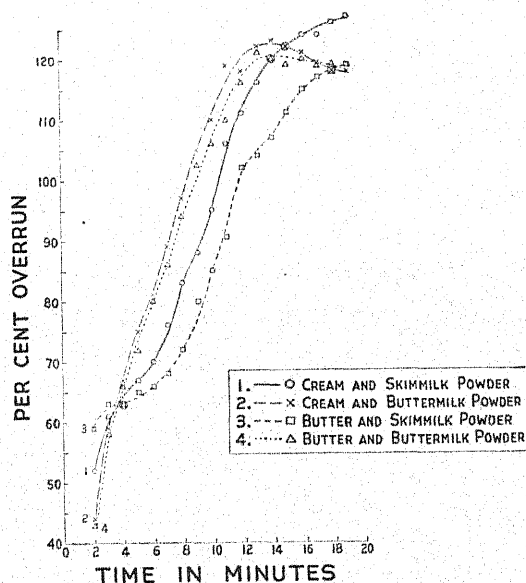


FIG. 1. The effect of roller process sweet cream buttermilk powder on the whipping ability of ice cream mixes containing cream and butter as sources of butterfat.

not-fat in the mix. Duplicate batches of each mix were frozen, and one batch was drawn at an overrun of 90 per cent, whereas the other batch was allowed to whip to the maximum overrun in order to obtain whipping ability data. The data obtained from the trial are presented in table 1 and figure 1. There were no marked differences among the viscosities of either the fresh or aged mixes in this trial. The most marked difference was evident in the rates of whipping of the mixes in the freezer. In each comparison (table 1) the mix which contained buttermilk powder attained an overrun of 90 per cent more rapidly than the skimmilk powder control, resulting in a saving in time of from about 2 to 3 minutes. In each case, it will be noted that the ice cream which contained buttermilk powder was drawn from the freezer at a lower temperature than the control. A more complete

picture of the whipping ability of the mixes is presented in figure 1. It will be noted that there was an initial "lag" in the rate of overrun development in the early stages of whipping for each of the buttermilk mixes. The greatest maximum overrun was attained in the cream and skimmilk powder mix. In each case, the ice cream which contained buttermilk powder was firmer

TABLE 1

Trial A.—The effect of sweet cream buttermilk powder upon certain properties of ice cream mixes and ice cream containing either cream or butter as the source of butterfat

Properties of ice cream mix	Mix			
	1	2	3	4
	Cream and skimmilk powder	Cream and buttermilk powder	Butter and skimmilk powder	Butter and buttermilk powder
Titratable acidity (%)	0.190	0.190	0.195	0.195
pH	6.31	6.31	6.32	6.31
Original viscosity (centipoises)	34.5	35.5	34.8	35.3
Apparent viscosity (centipoises)	179.0	144.0	147.5	155.0
Basic viscosity (centipoises)	55.3	45.3	48.5	49.5
Freezing time (min.: sec.)	2: 0	2: 0	2: 0	2: 0
Whipping time (min.: sec.)	7: 5	4: 16	6: 55	4: 51
Final overrun (%)	90.0	90.0	90.0	90.0
Temperature when drawn (° F.)	24.44	23.81	24.44	23.99
Condition when drawn	Sl. soft, wet	Very firm, dry	Sl. soft, wet	Firm, sl. wet
<i>Melting properties at 75° F.</i>				
Per cent drainage				
After 60 minutes	23.6	25.3	29.7	25.4
“ 90 minutes	56.8	57.0	63.9	54.7
“ 120 minutes	74.2	77.7	84.3	76.6
Appearance of meltdown	Coarse foam	Fine foam	Coarse foam	Fine foam
<i>Flavor scores and criticisms</i>				
After one week	39.0	38.5	39.0	38.0
	Lacks freshness, sl. cooked	Lacks freshness	Lacks freshness, sl. cooked	Lacks freshness
After four weeks	38.0	38.0	38.0	38.0
	Stale	Stale	Stale	Stale
<i>Body and texture scores and criticisms</i>				
After one week	28.0	29.0	28.0	29.0
	Coarse	Sl. coarse	Coarse	Sl. coarse
After four weeks	28.0	28.5	28.0	28.5
	Coarse	Coarse	Coarse	Coarse

and drier in appearance when drawn from the freezer than the control. The results of the meltdown tests indicate that there were no marked differences in the rates of drainage from the melting samples. All samples were foamy, but the foam from the samples which contained buttermilk powder was noticeably finer in structure and more stable than that of the controls. The initial flavor scores of the ice cream samples which contained butter-

milk powder were slightly less than the controls, but no differences were apparent after four weeks in storage. A cooked flavor was initially present in the samples which contained skimmilk powder, but this flavor was not detected in the other samples. The buttermilk ice creams were judged better in body and texture than the controls at both the initial and final scorings.

Trial B. It has been conclusively demonstrated by various investigators (1, 4, 7, 8) that as the butterfat content of the cream churned is increased there is an increase in the phospholipid content of the resulting buttermilk. Trial B was therefore designed to determine the influence of variations in the phospholipid content of sweet cream buttermilk powders upon the properties of ice cream mixes and the frozen products. Roller process sweet cream buttermilk powders were prepared from buttermilks obtained by the churning of three lots of sweet cream which contained 25, 33, and 40 per cent butterfat, respectively. The lower testing creams were obtained by standardizing portions of a single lot of 40 per cent cream with skimmilk from the same separation. A quantity of the skimmilk was dried for use in the control mixes. Four ice cream mixes were prepared from cream, skimmilk, and either skimmilk or buttermilk powder as follows:

<i>Mix number</i>	<i>Source of additional milk solids-not-fat</i>
1 (control)	R.P.* skimmilk powder
2	R.P. buttermilk powder from 25 per cent cream
3	R.P. buttermilk powder from 33 per cent cream
4	R.P. buttermilk powder from 40 per cent cream

* Roller process.

The data obtained from one set of mixes are presented in table 2, and the results of the whipping ability tests obtained from the freezing of duplicate batches are illustrated in figures 2 and 3. As shown in the table, the original, apparent, and basic viscosities were quite uniform for the entire series of mixes. Every mix which contained sweet cream buttermilk attained the desired overrun of 90 per cent in approximately 2 minutes less time than the control. An examination of the overrun-time curves of figure 2 reveals that the mixes attained practically the same maximum overrun. An initial lag in the overrun curves of the buttermilk mixes is clearly evident. The overrun-temperature relationships for each of the mixes are illustrated in figure 3. It is evident that the overruns of the mixes which contained sweet cream buttermilk powder were attained at a lower temperature than the control throughout the major part of the whipping process. As was found true in other trials, the freshly drawn ice creams which contained buttermilk powder were firmer and drier in appearance than the ice cream which contained skimmilk powder. The melting properties, flavor scores, and body and texture scores of the ice cream samples were quite uniform. The foam from the melting samples which contained buttermilk powder was finer in structure and more stable than that of the control sample. A cooked flavor was noted in the control sample but was absent in all other samples.

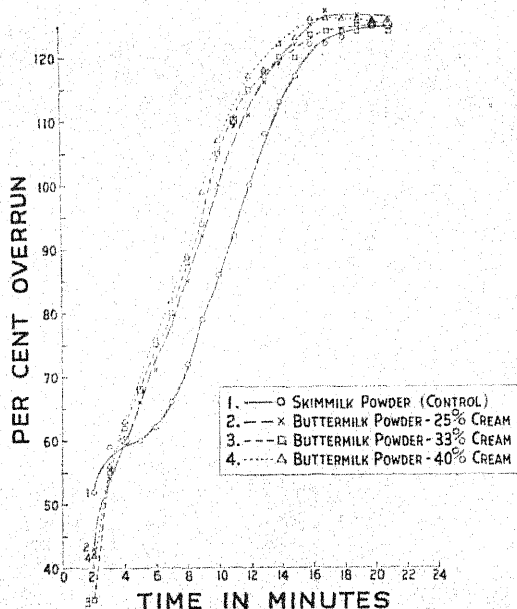


FIG. 2. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the whipping ability of ice cream mixes containing cream as the source of butterfat.

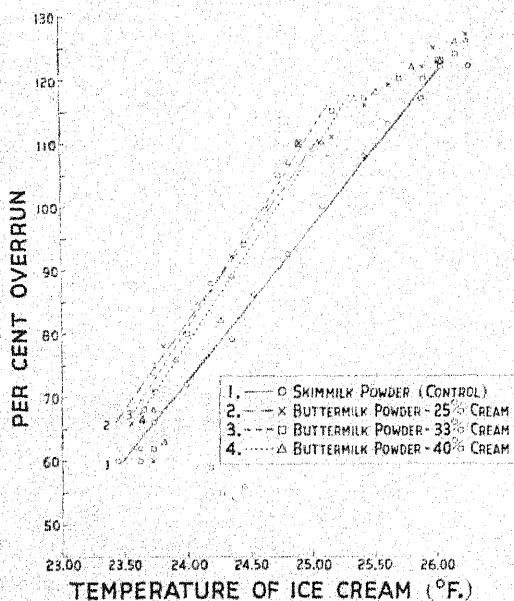


FIG. 3. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the overrun-temperature relationship of ice cream mixes.

Trial C. Trial C was conducted in exactly the same manner as trial B with the exception that butter was used as the source of butterfat instead of cream. The data presented in table 3 show that the results obtained practically paralleled those obtained in the previous trial. The viscosity data were more irregular, but the differences were not considered significant. It will be noted that the drainage from the melting ice cream samples which con-

TABLE 2

Trial B.—The effect of sweet cream buttermilk powders derived from creams of various butterfat contents upon certain properties of ice cream and ice cream containing cream as the source of butterfat

Properties of ice cream mix	Mix			
	1	2	3	4
	Skimmilk powder	Buttermilk powder*	Buttermilk powder†	Buttermilk powder‡
Titratable acidity (%)	0.190	0.185	0.185	0.190
pH	6.42	6.41	6.42	6.42
Original viscosity (centipoises)	42.5	44.0	43.3	44.0
Apparent viscosity (centipoises)	127.5	122.0	122.3	116.5
Basic viscosity (centipoises)	44.3	47.0	45.0	45.5
Freezing time (min.: sec.)	2: 0	2: 0	2: 0	2: 0
Whipping time (min.: sec.)	7: 40	5: 55	5: 50	5: 26
Final overrun (%)	90.0	90.0	90.0	90.0
Temperature when drawn (° F.)	24.62	24.35	24.26	24.26
Condition when drawn	Firm, sl. wet	Firm, mod. dry	Firm, mod. dry	Very firm, mod. dry
<i>Melting properties at 75° F.</i>				
Per cent drainage				
After 60 minutes	19.4	20.1	21.7	19.2
“ 90 minutes	49.6	50.0	51.8	47.0
“ 120 minutes	69.4	71.3	73.1	71.7
Appearance of meltdown	Coarse foam	Fine foam	Fine foam	Fine foam
<i>Flavor scores and criticisms</i>				
After three days	40.0	40.0	40.0	40.0
After three weeks	Cooked 40.0 Cooked	40.0	40.0	40.0
<i>Body and texture scores and criticisms</i>				
After three days	29.0	29.0	29.0	29.0
After three weeks	Sl. coarse 28.5 Coarse	Sl. coarse 28.5 Coarse	Sl. coarse 28.5 Coarse	Sl. coarse 28.5 Coarse

* Derived from 25 per cent cream.

† Derived from 35 per cent cream.

‡ Derived from 40 per cent cream.

tained buttermilk powder tended to take place at a slower rate than that of the control. The overrun curves presented in figure 4 show that the greatest maximum overrun was attained in the control mix. The differences in rate of overrun development were not as pronounced as in trial B, but otherwise the same general relationships existed. The overrun-temperature

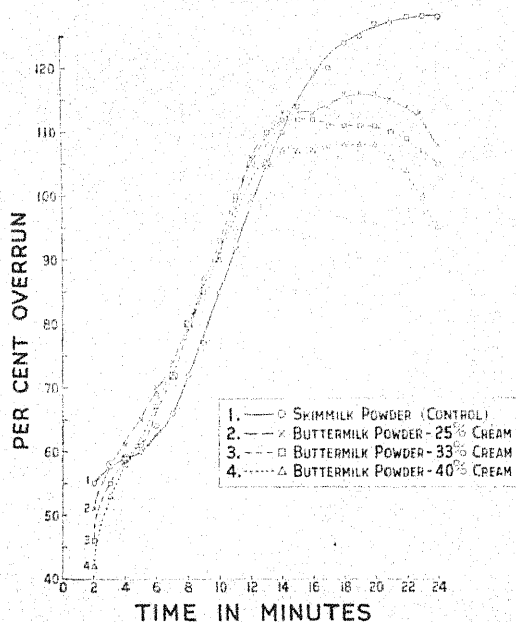


FIG. 4. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the whipping ability of ice cream mixes containing butter as the source of butterfat.

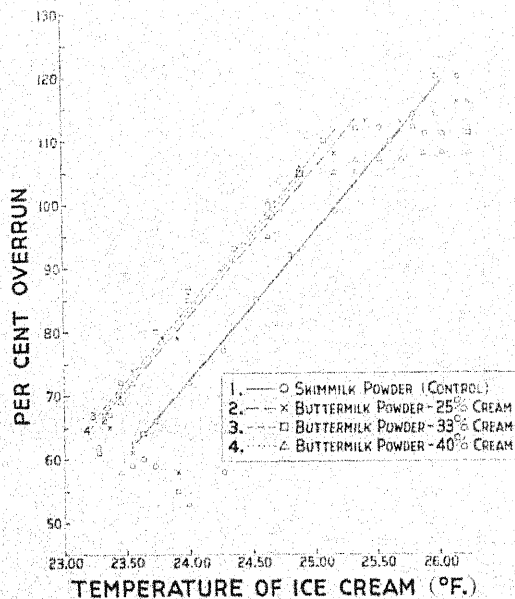


FIG. 5. The effect of roller process sweet cream buttermilk powders derived from creams of various butterfat contents on the overrun-temperature relationship of ice cream mixes containing butter as the source of butterfat.

relationships, illustrated in figure 5, show that the capacity to overrun at a given temperature was greater for the mixes which contained buttermilk powder than for the control throughout that part of the whipping process where a straight-line relationship existed. The use of Draw-Rite readings in comparing the consistencies of the ice creams throughout the freezing process served as evidence that each of the ice creams which contained sweet cream buttermilk powder was greater in consistency than the control throughout the major portion of the whipping period. The results are illustrated in figure 6.

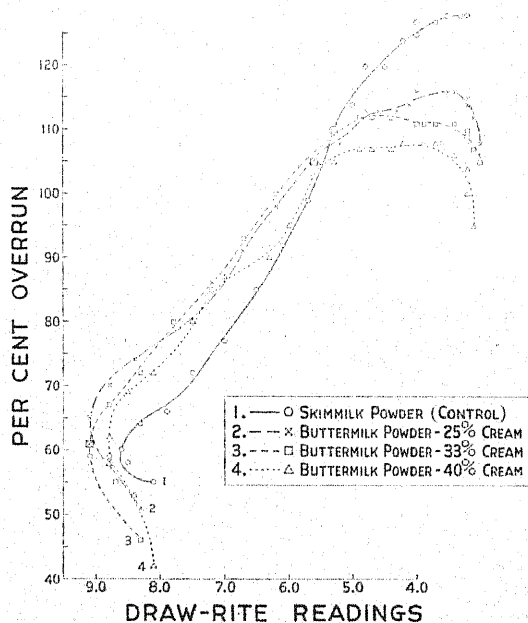


FIG. 6. The effect of roller process sweet cream buttermilk powders upon the consistency of ice cream mixes during the whipping process as measured by Draw-Rite readings.

SUMMARY

Experiments were conducted to determine the influence of roller process sweet cream buttermilk powder upon the properties of ice cream mixes and ice cream. The buttermilk product was used as the additional source of milk solids-not-fat for ice cream and the corresponding skimmilk product was used in the same manner in control mixes. In every comparison, the sweet cream buttermilk and skimmilk products were both derived from the same lot of mixed whole milk.

There were no consistent or marked differences in the viscosities of the ice cream mixes which could be attributed to any single factor.

TABLE 3

Trial C.—The effect of sweet cream buttermilk powders derived from creams of various butterfat contents upon certain properties of ice cream mixes and ice cream containing butter as the source of butterfat

Properties of ice cream mix	Mix			
	1	2	3	4
	Skimmilk powder	Buttermilk powder*	Buttermilk powder†	Buttermilk powder‡
Titratable acidity (%)	0.200	0.190	0.190	0.190
pH	6.35	6.33	6.34	6.34
Original viscosity (centipoises)	42.5	40.8	40.0	39.3
Apparent viscosity (centipoises)	133.0	160.5	118.5	105.5
Basic viscosity (centipoises)	52.0	45.0	44.0	40.5
Freezing time (min.: sec.)	2: 0	2: 0	2: 0	2: 0
Whipping time (min.: sec.)	8: 10	6: 45	6: 46	6: 0
Final overrun (%)	90.0	90.0	90.0	90.0
Temperature when drawn (° F.)	24.62	24.17	24.08	23.99
Condition when drawn	Sl. soft, wet	Mod. firm, mod. dry	Very firm, dry	Very firm, dry
<i>Melting properties at 78° F.</i>				
Per cent drainage				
After 60 minutes	43.4	34.1	32.8	38.1
" 90 minutes	72.3	61.8	59.9	61.9
" 120 minutes	92.4	85.5	84.2	84.6
Appearance of meltdown	Coarse foam	Fine foam	Fine foam	Fine foam
<i>Flavor scores and criticisms</i>				
After four days	39.5 Lacks freshness, cooked	39.5 Lacks freshness	39.5 Lacks freshness	39.5 Lacks freshness
After three weeks	38.5 Sl. cooked, lacks freshness	39.0 Lacks freshness	39.0 Lacks freshness	39.0 Lacks freshness
<i>Body and texture scores and criticisms</i>				
After four days	29.0 Sl. coarse	29.0 Sl. coarse	29.0 Sl. coarse	29.0 Sl. coarse
After three weeks	28.0 Coarse	28.0 Coarse	28.0 Coarse	28.0 Coarse

* Derived from 25 per cent cream.

† Derived from 33 per cent cream.

‡ Derived from 40 per cent cream.

A consistent improvement in the whipping ability of ice cream mixes was obtained through the use of roller process sweet cream buttermilk powder as the additional source of milk solids-not-fat in place of skimmilk powder. In most trials, the desired overrun was obtained in one to two minutes less time than for the control mixes. The superior whipping ability of the mixes which contained roller process sweet cream buttermilk powder was further evidenced by the fact that the desired overrun was obtained at a lower temperature than that of the controls. Overrun-time curves and overrun-temperature curves derived from whipping tests are presented

which show that the superior whipping ability of sweet cream buttermilk mixes persisted over a wide range of overruns. The maximum overrun obtainable was not increased through the use of roller process sweet cream buttermilk powder and tended to be somewhat inhibited in butter mixes.

Ice creams which contained sweet cream buttermilk solids were observed to be drier in appearance and greater in consistency when freshly drawn from the freezer than control mixes which contained skimmilk solids.

The melting resistance of the ice creams was not markedly affected by the use of sweet cream buttermilk solids. In all comparisons, it was noted that the ice creams which contained sweet cream buttermilk solids were more foamy in appearance during melting and that the foam of the filtrate contained smaller air cells than that of the control samples.

In many comparisons, the samples of ice cream which contained sweet cream buttermilk solids were judged as richer in flavor than the controls which contained skimmilk powder. It was further noted that whereas ice cream which contained roller process skimmilk powder was often criticized for possessing a cooked flavor, this criticism was never employed in evaluating the ice creams which contained roller process sweet cream buttermilk powder.

The use of roller process sweet cream buttermilk powders derived from buttermilk obtained by the churning of sweet cream testing either 25, 33, or 40 per cent butterfat was found to influence the ice cream mixes in a similar manner. In view of these results, it appears that the normal variations in the phospholipid content of buttermilk which result from the churning of creams within the above range of butterfat contents have no significant influence upon the properties of either ice cream mixes or ice cream, the maximum effect being approached through the use of buttermilk from 25 per cent cream.

CONCLUSIONS

The following conclusions are believed justified after a careful analysis of all observations relative to the experimental work reported above:

1. Neither the original viscosity nor the apparent and basic viscosities of the aged mix are significantly affected when using roller process sweet cream buttermilk powder in place of roller process skimmilk powder as the source of additional milk solids-not-fat for ice cream.
2. The use of roller process sweet cream buttermilk powder in ice cream mixes results in a greater rate of whipping than when using roller process skimmilk powder. Usually, 1 to 2 minutes less time is required to obtain a normal overrun, and the overrun is attained at a lower temperature. This is true when either cream or unsalted butter is used as the source of butterfat for the mix.
3. The use of roller process sweet cream buttermilk powder in ice cream results in a freshly frozen product which is drier in appearance and greater in consistency than when the skimmilk product is used.

4. Roller process sweet cream buttermilk powder tends to impart a richer flavor to ice cream than roller process skimmilk powder.

5. There is less tendency toward a cooked flavor in ice cream containing roller process sweet cream buttermilk powder than when containing the skimmilk product.

6. Ice cream containing roller process sweet cream buttermilk powder is characterized by a foamy meltdown, the foam being finer in structure and more stable than that of ice cream containing roller process skimmilk powder.

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THE ACTION OF THE MECHANICAL MILKER IN RELATION TO COMPLETENESS OF MILKING AND UDDER INJURY¹

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Although many are convinced that mechanical milking can be as good or better than hand milking, two major objections to machine milking are raised by some. One objection is that the mechanical milker causes injury to the teats and udder and the other is a claim that many cows will not milk out completely by the machine, necessitating milking varying amounts by hand.

The question as to whether or not the mechanical milker can cause injury to the teat and udder is an important one. It is common knowledge that trauma to the teat and udder, that is readily detected, is followed by a high incidence of clinical mastitis. Evidence is accumulating to indicate that trauma to the inner linings of the teat and gland, not detectable by palpation, may be an important predisposing factor to mastitis infection. Kennedy (3) has reported that by careful daily examinations of the teats and udder he detected trauma in varying degrees before the mastitis organism could be found in the milk.

It is well known that some cows do not milk out completely to the machine, as it is ordinarily operated. Sometimes as much as 40 per cent or more of the milk in the udder is removed by hand milking after removal of the machine. It is commonly observed that incomplete milking by the machine is more common among older cows than among first-calf heifers. It is obvious that much of the advantage of machine milking is lost when it becomes necessary to remove varying amounts of milk by hand milking.

THE PROBLEMS AND METHODS OF STUDY

Since a search of the literature failed to reveal any reports (except Petersen, 3) dealing specifically with either of these problems, work was instituted with a view of obtaining information toward their solution. Attempts were also made to compare the force exerted by the mechanical milker with that of hand milking.

To determine the action of the milking machine upon the udder a technique was developed whereby the excised mammary gland is used. The actual rate and completeness of milking by machine was ascertained on a number of cows by suspending the milking machine from a scale from which readings were taken every 20 seconds.

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The excised udder was suspended in the laboratory in approximately the normal position, by means of hooks in the median suspensory ligament and lateral facia. The gland cisterns were cannulated with glass cannula with a bore of 8 mm. or more. Skimmilk or physiological saline was then infused by gravity so as to maintain an intra-sinus pressure of approximately 50 mm. Hg. This is about the pressure developed in the cistern by the let down of milk. Skimmilk has been found more satisfactory than physiological saline as the latter will cause varying amounts of edema over a period of time.

Since the tonicity of the sphincter muscles surrounding the meatus of the teat is well maintained in the excised gland this preparation lends itself well to a study of mechanical milking. Observations have been made on the effect of such variables as pulsation rate, different vacuum levels, variations in size, shape and condition of the rubber inflations and others. Results of these observations will be presented in a later paper.

The most striking suitability of this preparation, however, was in the study of the action of the milking machine upon the teat and udder. After the gland had been filled with fluid through the inserted cannula the position of the teat cup on the teat could be observed with varying intra-sinus pressures, as could also the rate of milk withdrawal. The rate of inflow was regulated by adjustment of screw clamps on the rubber tubing connecting the skimmilk or physiological saline reservoir with the cannula in the sinus.

The action of the mechanical milker upon the inside of the teat and gland sinus was observed through incisions made in the lateral wall of the sinus or through a 2-inch tube inserted in an incision made perpendicularly over the orifice between the gland sinus and the teat. From the lateral incision the action could be felt by the insertion of a finger and actual measurements of the amount of suction developed within the teat were made by means of a vacuum gauge.

The force or "massage" action of the collapse of the rubber inflation upon the teat upon release of vacuum in the teat cup shell was also measured by the insertion of a small rubber bulb, attached to a manometer, into the teat sinus.

All of the observations reported herein were made by the use of double action mechanical milkers.

The measurements of the force applied to the teat in hand milking was accomplished both on the excised gland and on cows. In the excised gland this measurement was obtained by inserting a small rubber bulb attached to a mercury manometer into the teat sinus and different experienced milkers were used. The measurements on cows were taken with the same instrument, the rubber bulb being held in the palm of the hand so that pressure on the teat would cause compression thereof.

After a number of trials it was found that a rubber bulb of 2½ cc. capacity was the most satisfactory. This size held the minimum amount of mercury

needed and at the same time offered less interference to milking than larger bulbs. The bore of the manometer was such that it held 1 cc. mercury per 20 inches.

RESULTS

When the intra-gland sinus pressure remained constant in the excised gland the rate of milk withdrawal by the machine was constant for any given vacuum. With reduced intra-gland sinus pressures, the effect upon the rate of milk withdrawal varied with different glands. In some cases the rate of milk withdrawal began decreasing with a drop to 30 mm. Hg pressure and in others no detectable change was noted, until the intra-sinus pressure dropped to 10 mm. Hg or less. At the time that the rate of milk withdrawal began to decline the teat cups were noted to crawl upwards (C, fig. 1) with each vacuum stroke. The rates of crawling and decrease in milk withdrawal varied with different glands. In general it was noted that the rate of decline of milk withdrawal was more rapid when the crawling was also more rapid.

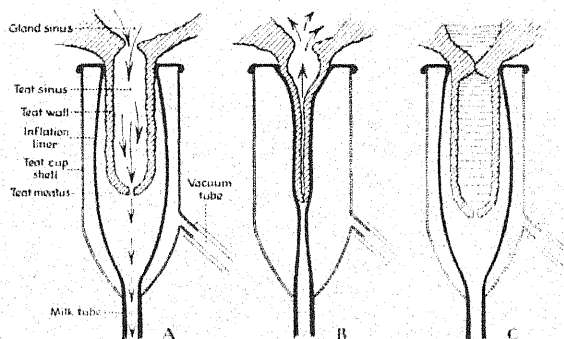


FIG. 1. Illustration of the action of the mechanical milker upon the teat. A. Vacuum applied in teat cup shell when the gland is full of milk. The teat meatus is opened and milk streams through it. B. Release of vacuum in the teat cup shell. The teat meatus is closed and a part of the milk in the teat sinus is forced back as a result of compression of the teat caused by the collapse of the inflation liner. C. Teat cup has crawled upward. The orifice between the gland and teat sinus is closed preventing passage of milk into the teat.

In 12 out of 14 cases the milk flow stopped before the gland was completely evacuated even though the machine was left attached. In all cases the gland was completely evacuated when the teat cups were pulled down part way on the teat. Once the teat cups had been permitted to crawl upward to a point where milk withdrawal had stopped it was necessary to increase the intra-gland sinus pressure by inflow of fluid to a point greater than that which would prevent the teat cup from crawling before milk withdrawal would again begin without a downward pull on the teat cups. While these values varied for different glands representative values lie between 10 and 20 mm. Hg greater pressure required to resume milk flow than is necessary to prevent the teat cups from crawling.

The reason for the teat cups crawling and the ultimate complete stoppage of milk withdrawal when the intra-sinus pressure is sufficiently reduced is seen through an incision through the sinus wall. By inserting the finger it was possible to ascertain that when the intra-gland sinus pressures were sufficiently high the orifice between this sinus and that of the teat remained wide open at all times as illustrated in A and B in figure 1. Under these conditions there is no restriction to the milk flow downward through the teat meatus upon the application of vacuum. It could also be felt that upon release from vacuum a small but definite spurt of milk flowed from the teat into the gland sinus as the result of compression of the rubber inflation upon the teat (B, fig. 1).

When the teat cups began to crawl upward as the result of reduced intra-gland sinus pressures the orifice could be felt to close. In most cases even though the gland sinus was filled with milk the closure was complete. In five instances free incisions were made so that the gland sinus communicated with the atmosphere without any air being drawn into the teat. If after the orifice between the gland and teat sinus is closed the teat cup is lowered part way down on the teat it was again opened.

Through the tube inserted perpendicularly through the gland the action of the pulsations on the tissues could be observed. It was noted that with each vacuum stroke the soft tissues surrounding the juncture of the gland and teat sinus were not only brought forcibly together but were also vigorously forced downward.

No detectable vacuum is developed in the teat as the result of milking machine action until there is partial closure of the orifice between the gland sinus and the teat. When the orifice is completely closed the vacuum within the teat sinus is the same as that in the milk line. With each release of vacuum in the shell of the teat cups the vacuum disappears within the teat because of the collapse of the rubber inflation compressing the teat.

It is difficult to obtain accurate measurements of the force exerted upon the teat by the collapse of the rubber inflation caused by the release from vacuum in the teat cup shell. The hammer-like force of the collapse has a tendency to cause the mercury, in the case of the mercury manometer, or the needle in the case of an air pressure gauge to overshoot. The values as observed are therefore only approximate. That there is a definite massaging action is certain. The approximate force is from 25 to 60 mm. Hg. Variations in the amount of force exerted were observed between different inflations of the same type, the reasons for which are obscure.

In a subsequent report factors affecting the speed of milking will be dealt with in more detail. Here will be presented only those observations on milking by machine that contribute directly to the action of the machine. In figure 2 is presented the results of milking a cow, first with the machine left on until no more milk was obtained, then followed by hand milking and

secondly, when the teat cups were drawn part way down at the time the rate of withdrawal began to decrease. It will be noted that the rate of milk withdrawal was constant for the first 2 minutes after which it decreased rapidly, with no manipulation of the machine, stopping completely in about 4½ minutes. After removal of the machine 2.1 pounds of milk were obtained by hand milking. When the teat cups were pulled partly down on the teats the rate of withdrawal did not begin to decrease until after 2 minutes, 40 seconds, and the gland was completely emptied without hand milking in 3½ minutes.

Without going into detail it must be stated that great variation exists between different cows in the type of curve presented by plotting rates of

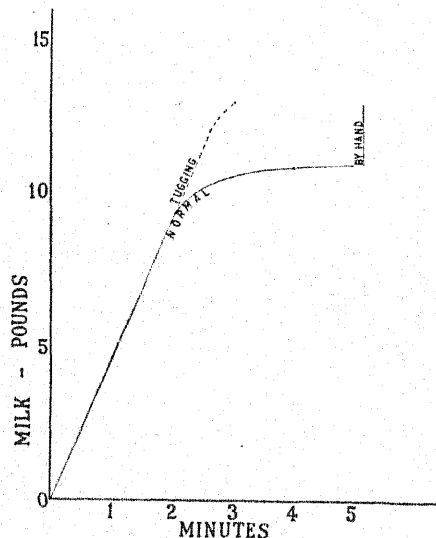


FIG. 2. Illustration of the effect of tugging upon the teat cups, when they begin to crawl, upon the rate and completeness of milking.

milk withdrawal by machine. So far, however, no cow in the University herd cannot be completely milked out by machine. For the majority of cows it is necessary to draw the teat cups downward to effect a complete evacuation of all of the milk in the gland.

The attempts at determining the force exerted upon the teat in hand milking was found to be difficult and not too satisfactory. The presence of the rubber bulb either in the teat sinus, in the excised gland, or in the palm of the hand in natural milking undoubtedly contributed to changes in the force applied. The inertia of the mercury is another factor contributing to error in the results that must not be overlooked. The values observed, therefore, must not be taken as absolute but merely indicative of the force exerted.

Measurements taken on four milkers varied from 16 inches to 24 inches of Hg pressure on the same cow. One fast milker exerted as much as 32 inches of Hg pressure in milking a hard-milking cow.

DISCUSSION OF RESULTS

The observations on the action of the mechanical milker upon the teat and udder in relation to varying intra-gland sinus pressures explains the observed curve characteristics when the rate of milk withdrawal is plotted. It is also in accordance with what would be expected on the basis of known physical laws. As all of the observations reported herein were made on the double action mechanical milker there is a continuous vacuum on the milk tube and alternate vacuum and release in the teat cup shell. The application of vacuum in the teat cup shell draws the rubber inflations toward the teat cup walls, permitting the full force of the continuous vacuum in the milk line to act upon the teat. Since this force is exerted equally in all directions, there is a tendency for the teat to be pulled down into the teat cup. This pulling down of the teat and lower part of the udder is inhibited by two other factors—the rigidity of the tissues as a result of the pressure of the milk within the gland and a partial displacement of the void by the outrushing milk through the meatus.

As milk withdrawal progresses the intra-glandular pressure is reduced and the tissues offer less resistance to the sucking action and are thus drawn farther into the teat cup. As the tissues at the juncture of the teat and gland sinuses are drawn into the teat cup the orifice between the two becomes occluded and further flow of milk from the gland is first decreased and finally completely stopped. When this occurs it is obvious that the full force of the vacuum is applied to the drawing in of the teat and lower part of the udder. Since the teat meatus is opened it is to be expected, as was found, that the vacuum within the teat is identical with that in the milk line.

In comparing the force of mechanical and hand milking it is significant that normal good hand milkers exert a greater force upon the teat than does mechanical milking with the usual vacuum. Also that the force exerted in hand milking is continuous throughout the entire milking process while in mechanical milking there is no measurable force exerted on the inside of the teat until the rate of milk withdrawal is reduced or completely stopped. It is possible that mechanical milking may have a greater traumatizing effect because of a somewhat shearing action than the straight compression action of hand milking. However, it is possible to so manage milking by machine, by removing it when free flow of milk ceases, that less injurious action will result to the teat and udder than with hand milking. On the other hand, if the machine is left on for prolonged periods after milk ceases to flow it can be injurious.

A well-established but little-recognized anatomical fact is the location of accessory secreting glands (4) in the gland sinus and in the upper part of

the teat sinus. These accessory glands have but a single layer of columnar epithelial cells as contrasted to the two layers of pavement epithelium lining the major portion of the teat sinus and are therefore more easily injured. The greatest compression in hand milking, particularly, in stripping and the most vigorous action of the milking machine is on this vulnerable area.

Upon the release from vacuum the rubber inflation collapses to mildly compress the teat and thus help prevent congestion. Before the teat is compressed the meatus closes leaving the teat sinus full of milk. Any further compression on the teat causes milk to be forced upward into the gland sinus. Since the compression of the teat is not complete not all of the milk in the teat sinus is forced back.

Variations in the point at which the teat cups begin crawling and in the amounts of milk left in the gland after milk withdrawal (without force applied to the teat cups) can tentatively be explained on an anatomical basis. Where the anatomical structure is such as to offer greater resistance to being drawn into the teat cup it is obvious that milking will be more complete before there is occlusion between the teat and gland sinuses. Great differences have been observed in the extent of the annular ring at the juncture of the teat with the gland sinus. In some cases this annular ring may extend more than half way across the orifice from one side to conceivably help cause an earlier occlusion during milking.

The observation that once the teat cups have crawled upwards to occlude passage from the gland sinus to the teat considerable increase in intra-gland sinus pressure is needed to again resume milk withdrawal explains in part why more rapid milking is obtained when the cow has been stimulated to let down milk before milking. Another factor is that unless let down has taken place the gland sinuses are emptied and the machine is acting for varying lengths of time without removing any milk before they are again filled. Ely and Petersen (1) reported that about 45 seconds intervened between the secretion of the hormone responsible for the let down of milk and the actual let down. In practice, therefore, it is suggested that the stimulation for the let down should take place about one minute before attaching the mechanical milker.

SUMMARY AND CONCLUSIONS

1. A technique is described whereby the excised udder may be used in the study of milking by mechanical means and results of observations of the action of the mechanical milker on both excised and intact glands are reported.

2. When milk flows freely from the gland sinus into the teat there is no detectable vacuum created within the teat sinus.

3. When the intra-glandular pressure is sufficiently reduced the tissues become more flaccid and the teat cups crawl upward to cause a complete

closure of the orifice between the teat and gland sinuses. This fact accounts for the reported incomplete milking of some cows by machine.

4. Tugging upon the teat cups, when they begin to crawl, with sufficient force to bring them part way down on the teats will prevent closure of the passage and permits complete evacuation of all of the milk in the gland without resorting to hand milking.

5. When the teat cups have crawled upward to close the passage from the gland sinus to the teat the vacuum within the teat becomes identical to that in the milk line. It is postulated that this will have a traumatizing action upon the tissues being compressed. Attention is directed to the location of accessory secreting glands in the gland sinus and often in the upper part of the teat sinus. These are easily injured which predisposes to mastitis.

6. Good hand milkers apply a greater force to the teat with each squeeze than does the milking machine at ordinary recommended vacuums.

7. Observations of the action of the milking machine upon the teat and udder explains why milking speed is increased by stimulating the cow to let down milk before the milking is started.

8. It is concluded that when the mechanical milker is properly operated, especially removed as soon as the milk ceases flowing there is less danger of injury to the teat and udder than from hand milking.

ACKNOWLEDGMENT

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THE EFFECT OF THYROIDECTOMY ON LACTATION IN THE BOVINE¹

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Although the galactopoietic effect of thyroxine or dried thyroid gland is well established, reports on the effects of thyroidectomy are conflicting. A significant decrease in the milk yield of thyroidectomized goats was reported by Grimmer (5) and Trautman (14), while Hibbs *et al.* (6) obtained lactation for more than a year following thyroidectomy. Nelson and Tobin (11) and Nelson (10) have reported obtaining no evidence that lactation in thyroprived rats were impaired, while Folley (2) observed marked diminution of milk secretion and subnormal subsequent lactations. More recently Preheim (12) found only a slight decrease in lactation in the thyroidectomized rat, while Karnofsky (7) reported a marked reduction. For lactating cows, Graham (4) observed a slight decrease in milk production following thyroidectomy.

In view, therefore, of the conflicting reports of various workers, investigations were undertaken to obtain additional information regarding effects of thyroidectomy on lactation.

PROCEDURE

Four grade Holstein females were selected for experimental subjects. Table 1 shows the age and previous history of each animal at the time of thyroidectomy.

TABLE 1
Experimental animals at time of thyroidectomy

Animal No.	Age (months)	History
A26	13	Virgin
A23	16	46th day, 1st gestation
A15	36	12th day, 2nd lactation
E294	45	7th day, 2nd lactation

In order to assure complete removal of the thyroid, exploratory operations were performed on A26 and A23 ninety days after the initial operation. Autopsy examinations confirmed complete thyroidectomy of E294 and revealed incomplete removal from A15. Blood analysis, general appearance and behavior also indicated that A15 retained some functional thyroid tissue throughout the experimental period.

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Parathyroid tissue embedded in the thyroid was unavoidably removed; however, the accessory parathyroids were left intact. No effort was made to determine the extent of the remaining functional parathyroid tissue.

Environmental factors such as care, management, housing and feeding were minimized to some extent by maintaining the experimental animals under the same conditions as the regular dairy herd.

Daily milk yield was recorded and samples taken for analytical purposes at 14 day intervals insofar as conditions would permit. These samples were analyzed for fat, total nitrogen, casein nitrogen, lactose and specific gravity.

RESULTS

The effects of incomplete and complete thyroidectomy on milk yield, fat yield and milk composition were studied. Data were accumulated on six lactations of four cows thyroidectomized at various stages of gestation and lactation.

Incomplete thyroidectomy. As shown in table 1 the thyroid glands were partially removed from A15 on the twelfth day of her first lactation period.

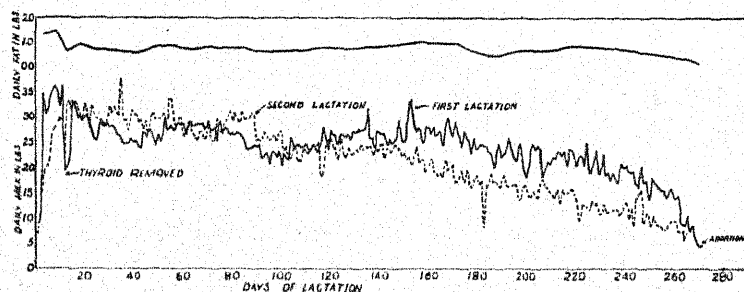


FIG. 1. Daily milk and fat yield of A15 following incomplete thyroidectomy on 12th day of first lactation. Second lactation began following abortion on 270th day of first lactation.

It is difficult to draw conclusions regarding the effects of incomplete thyroidectomy in view of the absence of previous lactations of A15 upon which to base a comparison. However, examination of the data presented in figure 1 reveals a distinct fall in milk production for 35 days following thyroidectomy, followed by a gradual upward trend of the curve of secretion. This response is undoubtedly explained, at least in part, by hypertrophy and hyperplasia of the remaining thyroid tissue which developed following the operation. The influence of thyroid hypertrophy is noticeably absent during the second lactation beginning after an abortion on the 270th day of the first lactation.

Milk yield. A comparison of the curves of milk secretion preceding and after thyroidectomy of E294, as shown in figure 2, clearly indicates the effects of thyroid ablation on milk yield. The point of maximum daily milk production was reached about 14 days previous to that of the normal lactation.

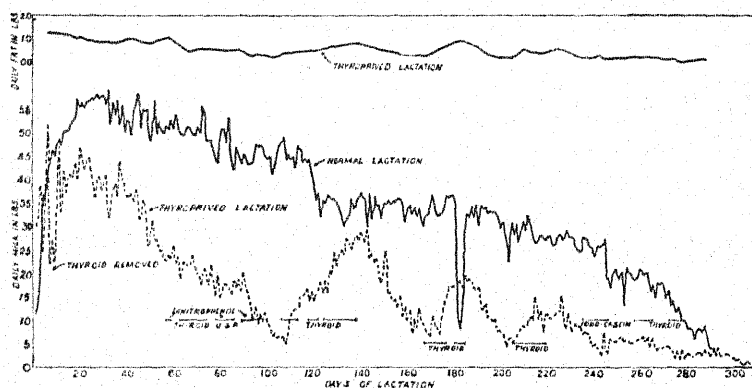


FIG. 2. Daily milk and fat yields of E294 for the lactation preceding thyroidectomy, for the subsequent thyroprived lactation, and during periods of thyrotherapy designated by —. Daily fat yields for prethyroprived lactation unavailable.

Milk secretion practically ceased after about 110 days while total milk yield was reduced about 70 per cent as compared to the previous lactations.

Milk secretion as affected by thyroidectomy during pregnancy is presented in figure 3. As shown in table 1, cow A23 was thyroidectomized on the 46th day of her first gestation which was of normal term. Unfortunately, two outbreaks of mastitis occurred during the lactation period; however, the general trend of the milk yield curve was not significantly altered. Daily milk production dropped to a low level after about 110 days and ceased entirely 183 days after parturition.

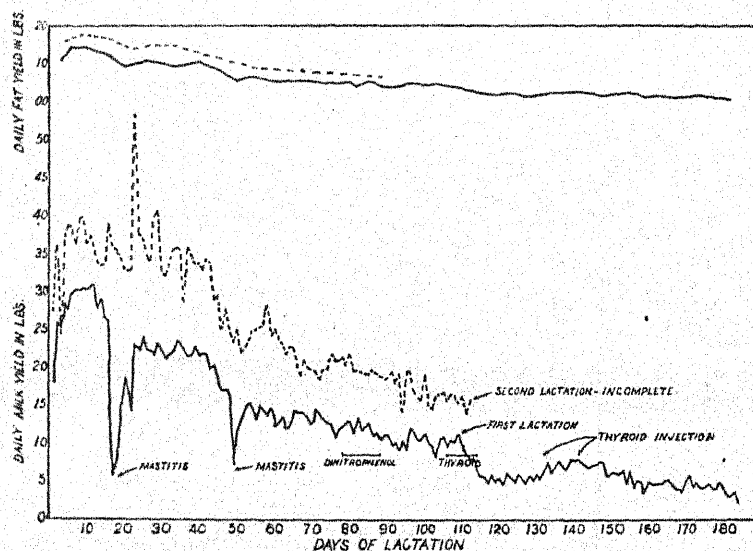


FIG. 3. First and second lactation milk secretion curves of A23 following thyroidectomy on 46th day of first gestation period.

Figures 1, 3, and 4 show the effects on milk secretion produced by removal of the thyroid glands before conception. The second lactation of A15 has been discussed. As shown in figure 3, A23 began her second lactation 720 days after thyroidectomy. A comparison of the second lactation and the preceding lactation indicates a higher level of secretion; however, the general trend of the curves is the same. Increased mammary development due to the beneficial effects of thyroid hormone or other factors from the developing fetus may account, at least in part, for the higher level of secretion observed during the second lactation. In this connection it is of interest to point out that a marked increase in skeletal growth of A23 was observed during the latter half of the preceding gestation period.

Figure 4 presents the milk secretion curve of A26 beginning 521 days after thyroidectomy. The curve of milk secretion is of normal shape; how-

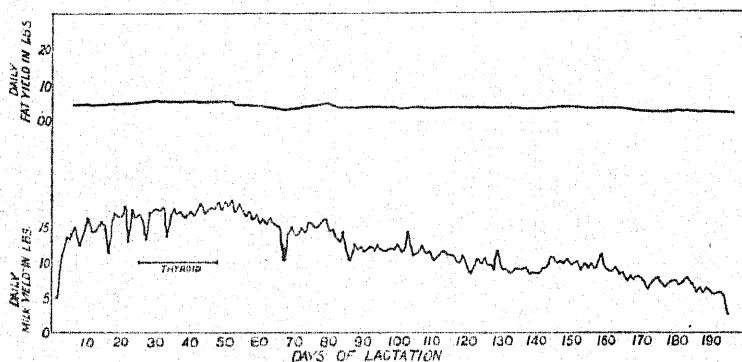


Fig. 4. Milk secretion curve of A26 beginning 521 days after thyroidectomy.

ever, lactation ceased 195 days after parturition. Although it is impossible to draw definite conclusions regarding the magnitude of the effects of thyroidectomy without previous knowledge of the producing ability of A26, it is significant that mammary development and milk secretion was possible some 521 days after thyroid removal.

Fat yield. The absence of more complete data on the daily production of milk fat precludes forming definite conclusions regarding the effects of thyroidectomy. It is, however, of interest to point out that based on the data obtained, the general trends and variations in fat yield were similar to those of milk yield.

Composition of milk. No significant changes in the lactose, total nitrogen and casein nitrogen content or specific gravity of the milk was evident from the results of periodical analyses. Values obtained for these constituents were all within the range for normal milk.

THYROTHERAPY

In view of the uniform decline of milk secretion observed after thyroidectomy, post-operative replacement therapy, in order to determine whether or

not some unknown non-endocrine factor was involved, seemed to be indicated.

Dinitrophenol. Dinitrophenol, administered orally to two thyroidectomized cows produced a variable response in milk and fat yield. Oral administration of 5 grams of dinitrophenol per day to A23 for 11 days beginning on the 78th day of lactation as indicated in figure 3 failed to evoke a response in milk secretion. The response of E294 to dinitrophenol administration was only slightly dissimilar, as shown in figure 2. During the period from the 78th day to the 87th day, 28 grams were orally administered producing an increase in milk yield of about four pounds per day. However, during the next period from the 89th to the 98th day, when 3.5 grams per day were administered, daily milk yield dropped from 16.6 to 9.5 pounds. As no calorimeter was available the relationship between metabolic rate, milk secretion and dinitrophenol administration is not known.

Thyroid. Fresh frozen thyroid glands were obtained from a local abattoir, mixed in a meat chopper, placed in gelatin capsules and administered orally. Desiccated thyroid, U.S.P. XI, was administered in two instances.

In order to ascertain whether or not thyroid deficiency was the sole limiting factor of maximum daily milk yield, 200 grams of thyroid were administered per day to A26 for 15 days beginning on the 23rd day of lactation. As indicated in figure 4, the response was negligible. This may be partially ascribed to progressive impairment of the normal physiological processes resulting in loss of ability to respond to thyroid therapy, as 546 days had elapsed since thyroidectomy.

Interpretation of results of thyroid therapy in the case of A23, figure 3, was complicated due to the presence of chronic mastitis.

The results of oral administration of fresh thyroid on milk and milk fat secretion are strikingly illustrated in figure 2. Fresh thyroid administered to E294 during six different periods, beginning on 106, 167, 198, 258, and 273 days of the lactation period, elicited a decreasing response with each trial until the increase in milk and milk fat yield was barely evident.

Iodinated casein. Turner (15) has presented evidence of the lactation-stimulating properties of iodinated casein. It was believed the availability of thyroidectomized cows presented an opportunity for testing the efficacy of this preparation. On the basis of response to previous administration of fresh thyroid, E294 possessed the ability to yield 12 to 14 pounds of milk per day. Oral administration of 3000 grams of iodinated casein over a period of 12 days resulted in only a slight increase in milk and milk fat yield. However, subsequent oral thyroid therapy failed to evoke a response; therefore, conclusions cannot be drawn regarding the lactation stimulating properties of iodinated casein.

In seeking an explanation for the decreasing response to thyrotherapy, progressive involution of the mammary gland with advancing lactation must

be considered. However, other factors may be involved, as involution of the mammary gland cannot account for the drastic decline and almost complete cessation of lactation 110 days after calving. Progressive impairment of normal physiological processes resulting in loss of ability to respond to thyroid therapy apparently occurs with the increasing interval of time between thyroidectomy and therapy.

DISCUSSION

Investigations on the rôle of the thyroid in lactation, using small laboratory animals, are hampered by the lack of suitable techniques for measuring milk yield, for microanalysis of milk, and the difficulty of achieving satisfactory and complete operative removal. These factors may partially account for the conflicting results of thyroidectomy studies reported in the literature.

Although control operations were not performed, post-operative recovery among the experimental animals, as indicated by daily milk yield, was uneventful and effected in about four days.

In view of the recent report of Folley *et al.* (3) that the failure of lactation in rats after thyroidectomy can be considerably alleviated by administration of parathyroid extract, cognizance must be taken of the parathyroids in thyroidectomy studies. Information regarding the location, number, and function of the parathyroid glands of the bovine is limited. Fehland (1) reported finding one parathyroid body embedded on the lateral surface of each thyroid lobe, and accessory parathyroids scattered along the course of the thyroid arteries. Although the amount of functional parathyroid tissue removed from or retained by each cow in this study was not determined, the absence of post-operative or parturitional tetany, characteristic of thyroidectomized rats, minimizes the importance of parathyroid insufficiency as a causative factor of decreased lactation. Normal blood calcium levels observed throughout the experimental period further substantiates the conclusion that our cows retained sufficient parathyroid tissue to suffice for normal calcium metabolism. Restoration of lactation by the administration of parathyroid extract to thyroidectomized cows would lend further proof.

Inasmuch as the administration of thyroid or dinitrophenol to thyroid-intact cows causes increases in fat content of the milk without necessarily affecting the quantity of milk, the absence of selective response of either milk or fat yield to thyrotherapy in this study is worthy of comment. These results may be explicable on the assumption that the metabolic level was lowered to such an extent that both milk and milk fat secretion would benefit from exogenous administration, whereas in the thyroid-intact animal selective response may occur. In this connection it is significant to point out that the milk of the thyroidectomized cows was of normal specific gravity and contained normal amounts of lactose, total nitrogen and casein nitrogen.

Results of replacement therapy strongly suggest that lactational failure observed in the bovine after thyroidectomy is due to thyroid insufficiency. However, the definite physiological effects of thyroid insufficiency causing decreased lactation are unknown. Subnormal mammary development in animals thyroidectomized a considerable period of time before pregnancy, as observed in this study, may be a factor. This observation is, however, contrary to those of Leonard and Reece (8) who reported increased mammary development after thyroid removal.

Karnofsky (7) and others, noting the changes in the pituitary gland after thyroidectomy, have postulated a decreased output of the lactogenic hormone as a limiting factor of lactation. McQueen-Williams (9) found that thyroparathyroidectomized male rat pituitaries contained less lactogen than normal. However, Reineke *et al.* (13) report both the lactogenic and thyrotropic hormones present in the pituitaries of thyroidectomized male kids in normal concentrations. Restoration of lactation to normal in thyroidectomized animals by administration of prolactin would confirm this postulation.

Milk secretion is dependent upon an adequate supply of metabolites from the blood. A decreased supply of metabolites may be due to lower levels in the blood stream or to a decreased flow of blood through the mammary gland. A slight reduction of heart rate in the thyroidectomized cows was observed. Reineke and co-workers (13) have suggested a reciprocal relationship between the thyroid and pituitary whereby removal of the thyroid results in a decrease of the pituitary factors regulating the metabolism of sugar, fat, protein, and mineral matter. Only in their presence can large amounts of milk be made. Normal levels of blood sugar, total nitrogen, calcium, phosphorus, and fat were observed throughout the experimental period for the thyroidectomized cows in this study.

Considerable stress is placed upon general metabolism during lactation. Inasmuch as thyroidectomy causes a lowering of metabolic activity this is undoubtedly a contributing cause of the rapid decline of milk secretion after thyroidectomy. Whether or not thyroidectomy results in a distinct lowering of metabolic activity of the individual secretory cells of the mammary gland remains a moot question.

SUMMARY

1. Thyroidectomy performed on cows preceding gestation, during pregnancy, and during lactation caused a complete cessation of lactation in about 180 days.
2. Total milk and fat yield were reduced about 75 per cent.
3. Incomplete removal of thyroid gland produced a temporary decline in milk secretion followed by a gradual return to former levels.
4. Composition of the milk as determined by fat, lactose, nitrogen, and specific gravity analyses was not affected.

5. Milk and fat yields were restored to former levels by oral administration of fresh thyroid. Decreasing response was observed as the lactation period progressed.

6. It is concluded that the reduction of lactation after thyroidectomy is due mainly to thyroid deficiency.

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THE EFFECT OF COMPLETE EVACUATION OF THE MAMMARY GLAND BY PITOCIN UPON MILK AND FAT PRODUCTION*

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It is generally recognized that incomplete removal of the milk from the udder over a period of time will cause a decline in lactation. Partial removal of the milk has been used by many as a means of "drying off" cows. Miller and Petersen (4) observed marked downward trends in the lactation curve when cows were stimulated to "let down" their milk 20 minutes before milking began, resulting in incomplete evacuation of the gland.

While the reason for this effect of incomplete evacuation of the gland is speculative at the present it can best be explained by increased intra-alveolar pressures created by the retained milk. Petersen and Rigor (5) and Garrison and Turner (3) showed a decreased rate of milk secretion with increased pressures. The former observed complete stoppage of milk secretion when these pressures reached 30 mm. Hg while the latter noted that 40 mm. Hg pressure was needed to completely stop milk secretion. The increased milk production observed with increased frequency of milking can also be explained by the hypothesis that this practice prevents the development of as great pressures or the maintenance of high pressures over as long periods of time as does less frequent milking.

Since it has been established that incomplete evacuation of the udder over a period of time will cause a drop in the lactation curve, the question arose as to what part natural incomplete emptying of the gland plays in the decline of lactation with the advance in lactation. A second question is that of the effect upon the lactation curve of cows that are erratic in their let down of milk and if incomplete let down of milk might not be the cause of the rapid decline in the lactation observed in many cases.

As the oxytocic principle (2), when injected intravenously, has been shown to practically completely evacuate the alveoli, use of this hormone following milking enables one to ascertain the amount of milk remaining in the gland after a normal milking has been completed. Complete evacuation of the gland at each milking, by use of this hormone, will also make it possible to determine the effect of this procedure upon the lactation curve.

EXPERIMENTAL

To determine the effect of complete evacuation of the gland at each milking upon the lactation curve in the declining phase of lactation 5 cows in

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advanced stages of lactation were used. The cows were mature grade Holsteins designated as E 293, E 366, A 12, A 15, and A 30. They were all normal during the period of the experiment.

The experiment was divided into three periods of fourteen days each. For E 293 and A 15, injections were made in the second period and the first and third periods served as controls. E 366, A 12, and A 30 were injected during the first and third periods and the second period served as a control.

All cows were milked twice daily at twelve-hour intervals. E 366 was milked by hand and the others by machine. In the case of machine milking completeness of milking was checked by hand stripping. The milk obtained by hand or machine plus any stripping is referred to as normal milk.

During the injection period 1 cc. Pitocin containing 10 I.U. of the oxytocic principle was injected intrajugularly immediately after the completion of the normal milking. The milk obtained following the injection, referred to in this experiment as "pitocin" milk, was weighed and analyzed separately.

In addition to the weights of milk obtained at each milking, lactose (1) chloride (6) and fat determinations were made.

The effect of complete evacuation of the udder by injection of Pitocin was studied on three cases that deserve special mention.

Case 1. A grade first-calf Holstein heifer that apparently did not let down her milk.

Case 2. A purebred Jersey in a nearby herd in the third month of her second lactation period, with a short time lactation history that could not be explained on a basis of heredity.

Case 3. A purebred Jersey cow in the fourth lactation that was unusually erratic in the let down of her milk.

RESULTS AND DISCUSSION

A summary of the actual milk and fat production and fat percentage by periods for both the "normal" and "pitocin" milks is presented in table 1. It is customary in experiments with the setup used here to calculate the effect of the experimental procedure by comparison of the experimental with the control periods. This has been done in table 1 but caution should be registered in accepting those values as representing the absolute effect of complete evacuation of the gland by Pitocin because nothing is known about the effect of the injections upon subsequent periods and all cows were in the declining phase of lactation with no means of knowing what the normal slope of the lactation curve would be. With the exception of A 30 the evidence seems to be in favor of the Pitocin injections causing a greater increase in production than indicated by the calculations. Because of the injections the subsequent control period seems to be at a higher level than it would have been normally. It can therefore be said that in every case

the complete evacuation of the udder by Pitocin injections causes a significant increase in the amount of milk produced.

The effect of the Pitocin injections upon the fat percentage is not so conclusive although the indications are that this value is also increased. In three of the cows there is the significant increase of fat percentage of 0.4+ while the decreases are 0.08, in both cases. It is to be noted that the "pitocin" milks are significantly higher in fat content than the "normal" milks. It is also noteworthy that the fat content of the "normal" milks during the injection periods are significantly lower than for the control periods.

TABLE 1

The effect of complete evacuation of the udder by Pitocin injections. Total milk and milk fat production and fat percentage by periods of 14 days each. For comparative purposes the milk production for a fourteen-day period previous to the start of the experiment was taken from the daily milk records. This is designated Control+.

Cow number	Experi- mental periods	Milk produced during 14-day periods						Period of in- jection as com- pared to control	
		Normal milking		Pitocin milk		Total			
		Milk	Fat	Milk	Fat	Milk	Fat	Lbs.	% fat
		<i>lbs.</i>	<i>%</i>	<i>lbs.</i>	<i>%</i>	<i>lbs.</i>	<i>%</i>		
E 293	Control+	452.3
	Control	399.9	3.29	399.9	3.29
	Injection	320.2	2.98	99.7	6.31	419.9	3.77	+ 62.2	+ 0.42
A 15	Control	315.5	3.41	315.5	3.41
	Control+	435.7
	Control	386.9	3.49	386.9	3.49
E 366	Injection	315.0	2.86	105.1	6.68	420.1	3.82	+ 45.8	+ 0.40
	Control	361.7	3.35	361.7	3.35
	Control+	456.4
A 30	Injection	363.6	2.84	102.0	7.16	465.6	3.79
	Control	410.2	3.34	410.2	3.34	+ 67.1	+ 0.43
	Injection	370.9	2.54	118.0	7.54	488.9	3.75
A 12	Control+	245.3
	Injection	229.0	3.58	24.1	7.68	253.1	3.97
	Control	234.5	4.06	234.5	4.06	+ 15.0	- 0.08
E 293	Injection	217.1	3.56	28.7	7.20	245.8	3.99
	Control+	149.3
	Injection	68.0	3.35	81.0	7.14	149.0	5.41
A 15	Control	31.7	5.39	31.7	5.39	+ 59.8	- 0.08
	Injection	19.9	3.37	14.0	7.81	33.9	5.20

While the production of both milk and fat in the experimental periods exceed that of the control periods by significant amounts for E 293, A 15, E 366, and A 30; for A 12 the production during the experimental period only equaled that of the control periods. This can be explained by the fact that she was in the last stage of the lactation and the drop in daily production was precipitous when the experiment was started.

The lactose and chloride contents of the milk were unaffected by Pitocin injections.

To ascertain the effect of Pitocin injections with time the total daily milk and fat production for all periods were plotted. For the injection

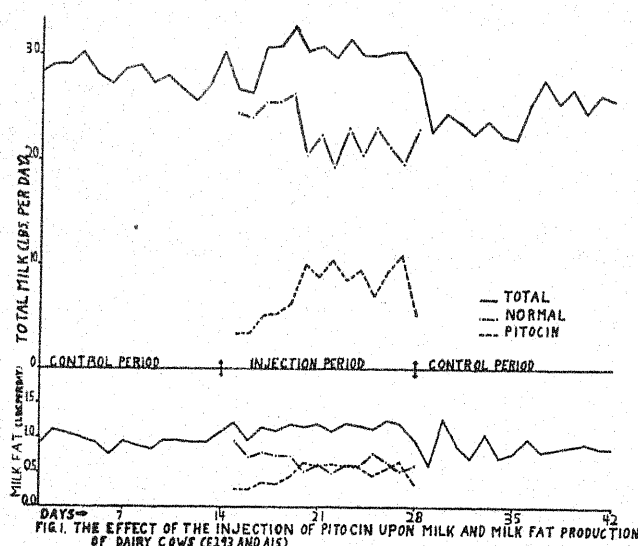


FIG. 1. The effect of complete evacuation of the udder by administration of Pitocin at each milking upon milk and milk fat production. The average daily milk and milk fat is plotted for E 293 and A 15.

periods the "normal" and "pitocin" milks and fats are also plotted. Because of the similarity of the curves these records of E 293 and A 15 are combined in figure 1 and for the same reason the records on E 366 and A 30

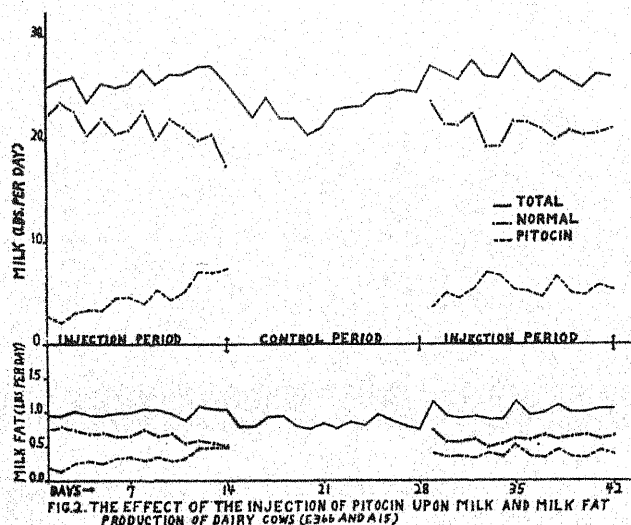


FIG. 2. The effect of complete evacuation of the udder by Pitocin administration upon the average daily milk and milk fat production on E 366 and A 30.

are combined in figure 2. The record of A 12 presenting a different picture is presented in figure 3.

As the injections continued during the 14-day periods, there was an upward trend in both total daily milk and fat production in spite of the fact that all of the cows were in decided declining phases of lactation. This trend is relatively the greatest in the second injection period in A 12 where also the drop was the most precipitous in the control period following the first injection period. This fact is taken as evidence that milk retained in the gland after normal milking is a contributory factor to the decline in production in late lactation. On the basis of the results on A 12 there is indication that "drying off" effect of the retained milk is the greatest in the most advanced lactation.

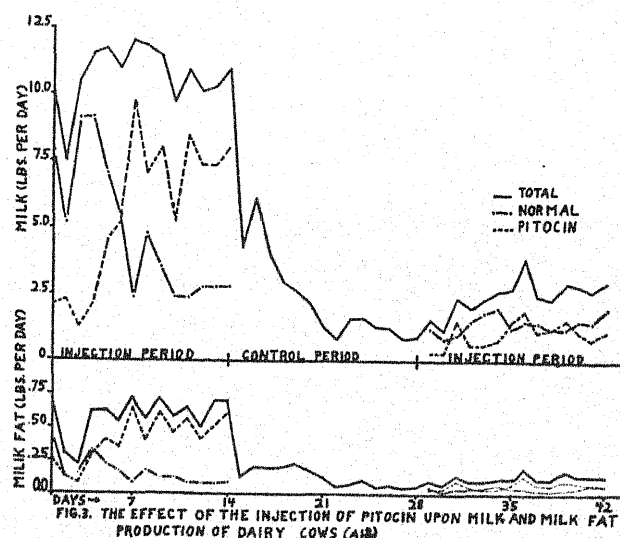


Fig. 3. The effect of complete evacuation of the udder by Pitocin administration upon the daily milk and milk fat production on A 12.

Another interesting observation is that as the injections continued there was a progressive decrease in the "normal" milk obtained and an increase in the "pitocin" milk and the shift in the ratio of the fat in the "pitocin" milk to that in the "normal" milk is even greater with the continuation of injections. This effect of Pitocin injections is the most prominent in the first injection period of A 12 (fig. 3) where in the whole period 68.0 pounds were obtained as normal milk and 81.0 pounds as "pitocin" milk while in the second half of the period the "pitocin" milk amounted to more than 3 times as much as the "normal" milk.

The reason for the observed phenomenon can only be speculative at this time. It is possible that the cows were becoming conditioned to the injection.

tions and did not respond as completely with the let down of milk to the normal milking. An alternate explanation is that the continued injections had a depressing effect upon the natural secretion of the oxytocic principle by the posterior pituitary body.

The results obtained by Pitocin injections into the 3 special cases investigated, adds confirmatory evidence to the depressing effect upon lactation of incomplete evacuation of the mammary gland even though such be from natural causes. Since each of the 3 cases present essentially different aspects of the problems they will be dealt with separately.

Case 1 can best be described as a first calf heifer that lacked the ability to respond with a "let down" of milk to the normal milking stimulus. The only milk obtained was that which had drained into the lower cavities of the udder and in the teats. She exhibited no nervous signs during attempts at milking and at no time could more than 3.1 pounds of milk be obtained at a milking in spite of the fact the size and appearance of the udder indicated a much greater producing capacity. After the small amount of milk was removed the lower part of the udder became "soft" but the upper two-thirds remained firm. After two weeks 1 cc. Pitocin was injected intrajugularly immediately after each milking and the additional milk removed. This procedure was continued for five days. During the injection period she averaged 16.2 pounds per milking of which 13.9 pounds were obtained following the injection of the hormone.

Upon cessations of the injections no more milk was obtained than during the first two weeks and at the end of two months milk secretion ceased. After evacuation of the gland following the injections it assumed the natural soft texture following the milking of an udder said to have good quality. The hardness of the gland was therefore due to the retained milk.

Case 2 differed from Case 1 in that she milked at normal levels after parturition but dropped off in production at a much more rapid rate than any of her closely related females in this herd. The case came to the attention of one of the authors when the owner explained the reason for the lack of persistency as being due to a "meaty" udder. She was then in the third month of lactation and was producing 9.5 to 10.3 pounds of milk per milking. After removal of this amount of milk the upper two-thirds of the udder remained hard or "meaty" as the owner had described.

Upon the intrajugular injection of 1 cc. Pitocin immediately after a normal milking of 10.1 pounds an additional 8.6 pounds of milk was obtained. Following the removal of this amount of milk the hardness or meaty condition of the udder had disappeared. Injections were continued by the owner for a period of 5 days with a reported increase of more than 50 per cent production. Upon cessation of injections she immediately returned to the former habit of incomplete evacuation of the gland.

As the amounts of milk obtained were far greater than the capacities of the cavities of the udder and teats and also as the amounts of milk obtained at milkings varied no more than could normally be expected it appears that in this case there was a uniform but incomplete response to the milking stimulus. Whether the lack of response was due to insufficient hormone secretion or to a decrease in the sensitivity of the gland musculature is conjectural.

Case 3 is a pure bred Jersey in the University herd now in her fourth lactation. Her history is of special interest and therefore her lactation curves for the first 3 completed and the 4th lactations to the present are given in figure 4. It will be noted that in her first lactation she was unusually persistent but in the subsequent ones the reverse is true.

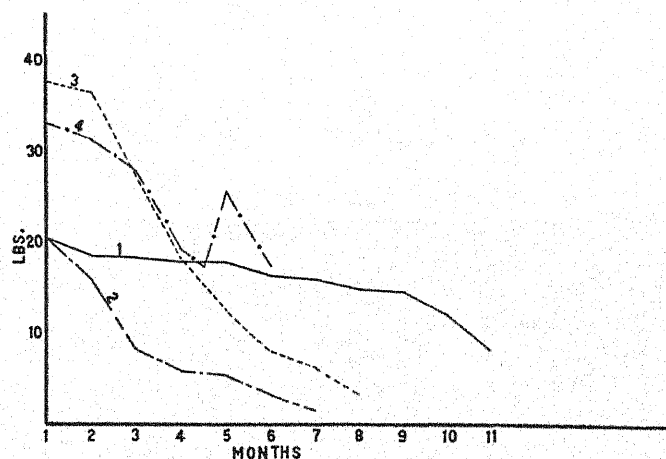


FIG. 4. The effect of erratic habit on let down of milk upon the character of the lactation curves, plotted by monthly averages except the portion of the fourth lactation where plotting is based upon weekly average. Successive lactations labeled 1, 2, 3, and 4. During the first lactation complete let down occurred at each milking.

Inspection of the daily milk records reveal that just before a marked decline appears in the lactation curves she becomes very irregular in the amount of milk obtained. It was not uncommon to obtain but two or three pounds of milk in a milking to be followed by one of fourteen to sixteen pounds. The variations in the month of her fourth lactation may be seen in figure 5. Following the milkings of small amounts the upper part of the udder remained hard while following the large milking it was soft and pliable. Inspection of the curve (fig. 5) for the first fourteen days reveals the probability that at some milkings there was apparently no response with a let down of milk while at others there was a partial response and sometimes a complete response. It is safe to say that the gland was completely

evacuated in less than one-third of the milkings, which fact was responsible for the rapid decline in her lactation curve.

For fourteen days (14 to 28 in figure 5) 1 cc. Pitocin was injected intrajugularly immediately after each normal milking and the milk let down removed. The milk obtained following Pitocin injection as well as the total milk for each milking is plotted in figure 5. It will be noted that the variations in the total milk obtained per milking was reduced to minor fluctuations. There were, however, marked variations from milking to milking in the relative amounts obtained as "normal" and "pitocin" milk.

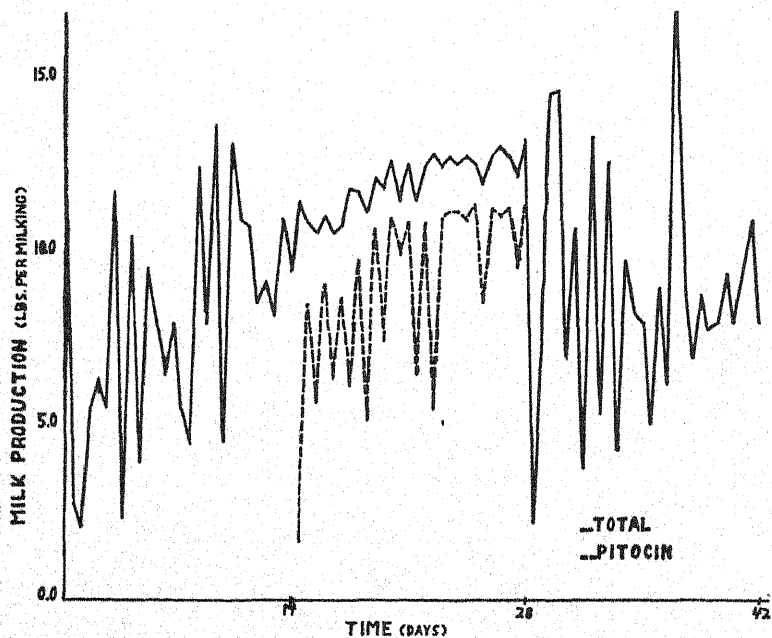


FIG. 5. The effect of complete evacuation of the udder by Pitocin injection upon the quantity of milk and uniformity of amounts of milk in a cow with erratic milking habits. Each milking is plotted. The first fourteen days is a control period. Pitocin was injected at each milking from the 15th to 28th day.

Using the milk production for the fourteen days immediately preceding the injection period there were increases of 44.8 per cent in milk and 59.31 per cent in fat for the injection period. It will be noted from figure 5, however, that the daily production increased for the first 8 days when a plateau is reached. Using the last six days of the injection period the production increased 56.9 per cent as compared with the average production for the fourteen days before injection.

As for the injections of Pitocin in cows in advanced lactation it is noted there is an increase in "pitocin" milk and a decrease in "normal" milk with

continuation of the injections. It is noteworthy that the decreasing effect of the Pitocin injections upon the amounts of milk lasted for but one milking after injections were stopped. It is also noteworthy that in the 14-day period following cessation of injections the milk production is 5.1 per cent greater than in the 14-day period preceding injections instead of an estimated 15 per cent loss as would be expected from the trend of the lactation curve.

It would therefore appear that the complete evacuation of the gland at each milking by the use of Pitocin not only checked the downward trend of the lactation curve but in this case stimulated the gland into greater activity.

SUMMARY AND CONCLUSIONS

1. The results are given of complete evacuation of the udder by the injection of Pitocin upon the milk and fat production of five cows in the declining phase of lactation and of three other cases of natural incomplete let down of milk.

2. In all cases of declining lactation complete evacuation of the gland checked the downward trend of the lactation. In all but one case the milk production was significantly increased over the control period.

3. On the basis of the results obtained on three cows with the evacuation of the gland by the injection of Pitocin it is suggested that in many cases the lack of persistency is due to the incomplete let down of milk.

4. Any milk retained in the gland has a depressing influence upon subsequent production.

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CHANGES IN BACTERIAL COUNTS OF STORED ICE CREAM MIX

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In ice cream surveys conducted by this station excessively high bacterial plate counts sometimes were obtained on samples frozen from mixes which originated at plants whose products ordinarily were of low bacterial content. Although sanitation in the freezing plant undoubtedly was a factor in some instances, other causes were indicated in a sufficient number of cases to warrant study of the effect of time and temperature of storage upon the development of bacteria in ice cream mix.

Abele (1) reported only 80 per cent of the mix in the hands of the manufacturers and 75 per cent of that in possession of the freezers was held below 50° F. Temperatures of ice cream mix in transit commonly were found to rise above 50° F., and sometimes to 60° F., accentuating the problem of adequate refrigeration during storage in the plant where the mix was frozen.

METHODS

Samples of pasteurized mix were obtained from two sources and at different seasons of the year over a period of 15 months. During this period ingredients and formulae were changed, especially at one source, so the samples represented a diversity of composition. Each original lot of mix was agitated thoroughly and approximately 50-ml. quantities transferred aseptically to 2-oz. screw-cap sample jars. A number of replicate samples sufficient to provide an undisturbed sample from each temperature of storage at each plating interval was employed. The samples were held under commercial refrigeration conditions, periodic temperature readings being recorded. On some samples the temperatures may have exceeded the recorded limits for short unrecorded intervals, but the ranges given represent the temperatures in effect during most of the holding period. All platings were made by using the volumetric method, standard plate counts being on Tryptone-glucose-extract-milk agar incubated for 48 hours at 37° C., and coliform counts being on violet-red bile agar incubated approximately 20 hours at 37° C. The procedures used were those outlined in "Standard Methods for the Examination of Dairy Products" (2).

RESULTS

The results of the bacteriological examinations made after various intervals are recorded in table 1. The data indicate some lots of mix cannot be

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TABLE 1
Changes in bacterial counts of ice cream mix during refrigerated storage

Sample No.	Holding temperature range (°C.)	Standard plate count per ml. after holding for periods indicated			
		0 days	2 days	4 days	7 days
1	2.0-10.0	9,300	4,200	17,000	28,000
	13.0-14.5	9,300	10,000	1,500,000	75,000,000
2	4.5- 5.5	14,000	13,200	9,100*	11,700
	7.0- 8.0	14,000	19,000	9,800*	16,000
3	4.5- 5.5	41,000	92,000	178,000*	3,400,000
	7.0- 8.0	41,000	185,000	8,800,000*	68,000,000
4	4.5- 5.5	2,500	4,500	2,300	23,000†
	5.5- 8.5	2,500	6,300	280,000	19,000,000†
5	12.8-14.5	2,500	9,700	1,500,000	47,000,000†
	3.0- 4.5	9,200	9,800	40,000	470,000
6	8.5- 9.5	9,200	35,000	920,000	30,000,000
	12.2-13.3	9,200	78,000	4,200,000	135,000,000
7	3.0- 4.5	15,000	6,100	6,600	51,000
	8.5- 9.5	15,000	7,100	150,000	9,500,000
8	12.2-13.3	15,000	10,000	1,300,000	57,000,000
	3.0- 4.5	6,600	4,600	11,500	116,000
9	8.5- 9.5	6,600	8,700	180,000	5,400,000
	12.2-13.3	6,600	13,400	940,000	16,100,000
10	1.0- 4.5	3,700	6,100	9,200	20,000,000
	4.0- 5.0	3,700	4,500	24,000	53,000,000
11	14.3-15.5	3,700	17,000	510,000	240,000,000
	3.0- 4.5	20,000	59,000	66,000	156,000
12	8.0- 9.5	20,000	115,000	7,000,000	61,000,000
	12.2-14.0	20,000	220,000	13,000,000	97,000,000
Coliform count per ml.					
1	2.0-10.0
	13.0-14.5
2	4.5- 5.5	1	1	0*	7
	7.0- 8.0	1	2	13*	5
3	4.5- 5.5	320	380	380*	700
	7.0- 8.0	320	920	3,400*	18,100
4	4.5- 5.5	1	1	< 1	1†
	5.5- 8.5	1	1	72	4,900†
5	12.8-14.5	1	6	> 100	710,000†
	3.0- 4.5	> 100	370	450	320
6	8.5- 9.5	> 100	450	410	160,000
	12.2-13.3	> 100	500	11,000	270,000
7	3.0- 4.5	> 100	440	380	220
	8.5- 9.5	> 100	590	630	500
8	12.2-13.3	> 100	800	900	1,500
	3.0- 4.5	1	1	1	300†
9	8.5- 9.5	1	4	7	420
	12.2-13.3	1	6	420	500
10	1.0- 4.5	< 1	< 1	< 1	< 1
	4.0- 5.0	< 1	< 1	< 1	< 1
11	14.3-15.5	< 1	< 1	< 1	< 1
	3.0- 4.5	120	70	10	130
12	8.0- 9.5	120	350	16,000	320,000
	12.2-14.0	120	700	59,000	1,600,000

* = 5 days. † = 8 days. ‡ = Questionable coliform types.

held more than 4 days at temperatures slightly below 5.5° C. (42° F.) without significant increase in count. No relationship between initial coliform count and tendency for standard plate count to increase at this temperature is apparent. In some instances relatively high counts were obtained after holding the mix 7 days at 5.5° C. (42° F.) or below. Temperatures of storage ranging up to 9.5° C. (49° F.) commonly resulted in some increase in standard plate count after 2 days and a large increase in count after 4 days. Holding mix at this temperature for 7 days usually resulted in excessive counts. Holding at temperatures approaching 15.5° C. (60° F.) sometimes permitted considerable increase in standard plate count in 2 days and always resulted in excessively high counts after 4 days.

Development of coliform organisms was checked by holding temperatures of 5.5° C. (42° F.) or below. At temperatures approaching 9.5° C. (49° F.) the behavior of this group of organisms was erratic, considerable increases in count being encountered in some samples, while these organisms multiplied but little or not at all in this temperature range in other samples. Storage at temperatures of 12.0° C. (53.6° F.) and above resulted in marked increases in coliform count, even after 4 days.

In no case had the flavor or aroma of the mix been changed significantly during the holding period of 7 or 8 days.

DISCUSSION

The data indicate storage of mix initially having a low bacterial count may permit considerable increases in "total" count. These increases are of particular significance when temperatures above 5.5° C. (42° F.) are encountered. Increases in coliform count usually occurred when temperatures reached the level of 8.0° C. (46.4° F.) or above, but were not significant below this temperature level. Increases in numbers of these bacteria during storage could result in erroneous conclusions concerning magnitude of post-pasteurization contamination of the mix.

The data indicate the practice of making or purchasing mix and storing it under moderate refrigeration for several days before freezing may have an undesirable effect upon the bacteriological condition of the resulting ice cream. Hammer (3) showed more than 30 years ago that the bacterial content of frozen ice cream fails to increase, in fact usually decreases, during storage. Storage in the form of frozen ice cream, rather than in the form of unfrozen mix, is to be preferred in either the manufacturing or the freezing plant. If the temperature can be maintained below 40° F., storage of mix for not more than 2 days apparently would cause no bacteriological difficulties under usual conditions.

CONCLUSIONS

1. The bacterial count of ice cream mix stored at 4.5° C. (40° F.) or

above increases with storage time and may reach considerable magnitude as the storage temperature increases.

2. Coliform bacteria usually were found to increase in numbers in ice cream mix held at temperatures of 8.0° C. (46.4° F.) or above. This increase may give rise to false conclusions relative to post-pasteurization contamination based upon presence of coliform bacteria in appreciable numbers.

3. Storage of frozen ice cream, rather than unfrozen mix, is recommended.

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A SIMPLIFIED METHOD OF ESTIMATING 305-DAY LACTATION PRODUCTION*

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Records of 305-day milk and butterfat production are ordinarily estimated from monthly milk weights and butterfat tests. Probably the most widely used method is that recommended by the Division of Dairy Herd Improvement Investigations, Bureau of Dairy Industry. The D.H.I.A. method has become known as the centering method because the testing month is centered around an established testing day rather than coinciding with the calendar month. In addition to this, the method involves calculation of back-credit in cases of lactation beginning at certain times in the testing month. In an investigation using D.H.I.A. records many errors in calculation were found. The majority of these errors were made in calculating the back-credit period, but many others resulted from the considerable multiplication and addition involved. This means that unless much time is spent in checking records, selection of animals is likely to be based on inaccurate information. Any method which might be substituted for the present D.H.I.A. system should be simple enough to result in greater arithmetical accuracy and should yield a record which possesses those statistical properties important in genetic selection.

The summation of the first ten testing-day values multiplied by 30.5 suggests itself as a simple method which should result in less arithmetical error. In this study the statistical properties of records calculated by this simplified method have been compared with the same cows' 305-day production records computed by the D.H.I.A. centering method and with actual production records obtained by summing the daily milk weights for the first 305 days.

LITERATURE

Prior to the advent of the cow testing associations, producing ability in dairy cattle had been estimated from records based on weekly butter yield and daily milk weights. Other estimates were based on seven-day tests in the fourth month multiplied by the number of weeks in the lactation; and still others by adding the milk weights for three days per month over a 12-month period and multiplying this sum by 10 to estimate yearly production.

Since cow testing associations were established, records have been calculated in most cases by the D.H.I.A. centering method. In some cases, how-

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ever, a calendar month scheme which is based on monthly tests and the number of days the cow is milked during the month has been used, as well as another method which estimates the production for the period between two consecutive testing dates from the milk and butterfat yields of the later date. Still other estimates have been derived from daily milk weights and a monthly or bimonthly fat test, and Plum (8) has used the summation of the first eight testing-day yields in an investigation of the causes of differences in butterfat records.

Studies by Rabild (9), McCandlish and McVicar (5), McDowell (6), Copeland (1), Gifford (2), Kendrick (4) and McKellip and Seath (7) indicated that little difference exists between the averages of production records estimated by these various methods. Harris, Lush and Shultz (3) found no significant differences in repeatabilities of D.H.I.A., lactation and yearly production records.

MATERIALS AND METHODS

The milk and butterfat figures used in this study were obtained from the D.H.I.A. herd record books and the daily milk records of two Wisconsin State Department of Public Welfare herds. These figures represented the production between 1935 and 1941 of Holstein cows milked three times daily. In one herd (Herd I), the butterfat records on 257 lactations of 108 cows and the milk records on 160 lactations of 60 cows were used. In the other herd (Herd II), the study was based on the milk records from 81 lactations of 65 cows. The milk records calculated by the D.H.I.A. centering method and simplified method were compared with each other and with the actual production figures obtained by summing the first 305-days' milk weights. Only the former comparison, "centered" vs. "simplified," could be made with the butterfat records.

Differences in the means of the records were tested for significance using Student's "t" test for paired observations. Simple correlations between estimated and actual yields were calculated. The variances of the milk and butterfat records, calculated for each herd by both methods, were divided into portions due to cow differences and differences between records of the same cow. The repeatability of milk and butterfat estimates (correlation between records made by the same cow) was computed for each class of records, *i.e.*, for the simplified, centered and actual production figures.

RESULTS AND DISCUSSION

The analyses show that the simplified and centered estimates are highly correlated with each other for both milk and butterfat production, and that the actual milk records are also closely associated with the two estimates of milk production (tables 1 and 2).

The means of the centered records for butterfat and milk production are significantly higher than the averages of the simplified records in Herd I

TABLE 1

Correlations between milk records calculated by the simplified and centering methods and actual yield in Herds I and II

Herd	I		II	
	Simplified	Centering	Simplified	Centering
Actual	0.990	0.992	0.984	0.991
Simplified	0.995	0.984

(tables 2 and 3). These higher means are probably caused in large part by the back-credit additions used in the calculations of centered records. The tester tests all milking cows that are more than six days beyond calving at

TABLE 2

Statistics of butterfat records calculated by the simplified and centering methods in Herd I

Method	Number of records	Mean butterfat production	Standard deviation	Mean difference	Correlation between	
					Simplified and centered records	Intra-cow records
Simplified ...	257	507.8 ± 6.3	101.2	3.4*	0.993	0.24
Centering	257	511.2 ± 6.3	100.8			0.25

* $P < 0.01$.

the time of his visit. Cows which both freshen and are tested during a testing period are given credit for the portion of the testing period they should lactate, less three days. Cows calving during an immediately previous testing period and not tested within that period are given credit (back-

TABLE 3

The actual, simplified and centered mean milk yields and differences between these means in Herds I and II

	Number of records	Actual	Simplified	Centering
Herd I	160			
Mean yield	14494 ± 207	14600 ± 210	14727 ± 212
Difference			
Actual		106*	233*
Simplified			127*
Herd II	81			
Mean yield	12683 ± 234	12769 ± 238	12565 ± 234
Difference			
Actual		86†	118*
Simplified			204*

* $P < 0.01$.

† $P \cong 0.03$.

credit) for the portion of the lactation which occurred in the previous period, less three days, on the basis of production during the present testing period. At each test (subsequent to the first) in the lactation of a cow, production is calculated for the testing period from the testing-day figures multiplied by the number of days in the testing period.

In a sense there are two groups of cows, one in which the cows start their records by receiving back-credit and another in which they do not. At the end of the record the "back-credit" cows will use only a part of the tenth testing period to complete a 305-day period. For the "non-back-credit" cows a portion of the eleventh testing period must be added to obtain an estimate of production over a 305-day period.

The simplified scheme also requires that each cow tested must have calved six or more days previous to the tester's visit, but no correction is made for day of calving (each testing-day value being multiplied by 30.5). Hence the simplified calculations give equal weight to the production on all of the first 10 testing periods, whereas the back-credit calculations give more weight to the higher production of the first testing period and less weight to the lower production of the tenth testing period than does the simplified scheme. Therefore, in "back-credit" lactations, the centered records are larger than those determined by the simplified method. On the other hand, cows receiving no back-credit will have lower centered records than simplified records because only a portion of the first testing period is used (the lower production of the eleventh period being used to complete the record), while in the simplified calculations equal weight is again given to all of the first ten monthly tests.

Furthermore, in the centering method the chances of cows receiving and not receiving back-credit are unequal. This grows out of the fact that a lactation record begins on the fourth day of lactation, whereas a test cannot be made until the seventh day of a lactation. Three days thus are added to the portion of the month in which a cow may calve and receive back-credit (average for different months of the year approximately $14\frac{1}{2}$ out of $30\frac{1}{2}$ days), and likewise the portion of the month in which they should not receive back-credit (about 16 out of $30\frac{1}{2}$ days) is lessened by three days. The two periods therefore approximate $17\frac{1}{2}$ and 13 days respectively. Thus the average of many centered records can be expected to be higher than the mean of corresponding simplified figures.

The simplified estimates of milk production in Herd II, on the other hand, are significantly higher than the centered records. The difference between Herd I and Herd II in these results seems to lie in the difference between these herds in regard to the deviations of actual testing dates from the established centering-day (table 4). In Herd I the deviations between the testing dates and centering-day are small, whereas the deviations in Herd II are large, varying from seven days before to 23 days after the centering-day.

Since the actual testing dates in Herd II were usually later than the fixed centering-date (ave. = + 7.8 days), the testing days were near the end of the testing period. This means that cows which would have received back-credit (i.e., freshened after the seventh day before the tester's visit) if the actual testing date had been closer to the centering-day would be included in the first testing period of that lactation, and therefore receive no back-credit. The increase of non-back-credit lactations in this herd undoubtedly explains why the centered records are lower than the simplified records in Herd II (table 3).

The means of the simplified estimates are significantly larger than the means of the actual milk yields in Herds I and II (table 3). One explanation for this is that the first test is frequently made at a time when the cow is producing at a higher rate than the average for that part of the first 30.5-day period in which she is actually in production. In addition, cows tested soon after freshening are given credit for production, in the period before

TABLE 4

Deviations of actual testing dates from the established centering-day in Herds I and II

Herd	No. of testing periods	Average deviation (days)	
		Arithmetic	Algebraic
I	72	1.9	-1.7
II	42	9.7	+7.8

they begin production, at a higher level than that which is used (after the tenth testing period) to complete the 305 days of actual yield.

The significantly higher mean of the centered records compared to the mean of the actual milk yields in Herd I (table 3) is caused, in part at least, by the centering scheme's back-credit calculations. Part of this difference between means arises from the large number of records of "back-credit" cows, in which the rate of production used to calculate back-credit is higher than the actual rate of production during this back-credit period. It also arises from the "non-back-credit" records in those cases in which the tester's first visit comes near to the peak of the lactation curve.

The data for Herd II show that the average of the centered estimates is lower than the mean actual milk yields. The consistently late testing dates in this herd are undoubtedly the explanation for this lower mean. That is, the frequency of the "back-credit" cows is reduced about half, and the production estimates for the centering periods during the declining phase of the lactation curve are based on testing day yields taken near the end of each period; at this time the yields are lower than the average actual daily yields for those periods.

The repeatabilities (intra-cow correlations) for the two estimates of butterfat and milk production and for actual milk production are given in tables 2 and 5. There is not a significant difference between the repeatabilities of the simplified and centered estimates of butterfat production, nor are there any significant differences between these repeatabilities for the simplified, centered and actual milk records within herds.

TABLE 5
Repeatabilities of simplified and centering methods and actual milk production records in Herds I and II

Herd	Number of cows	Simplified	Centering	Actual
I	60	0.36	0.35	0.38
II	16	0.55	0.53	0.52

SUMMARY AND CONCLUSIONS

A simplified scheme for computing 305-day milk and butterfat records has been described, and such records have been compared statistically with those estimates calculated by the D.H.I.A. centering method and with actual 305-day milk figures.

The high correlations found between simplified, centered and actual milk records and between simplified and centered butterfat estimates, and the close similarity of their intra-herd repeatability figures and mean yields for milk and butterfat production indicate no important differences between the simplified and centered schemes.

The simplified scheme would offer, however, the following advantages:

1. It would avoid most of the sources of those arithmetical errors which so often occur in the centered calculations.
2. It would facilitate the training of testing supervisors.
3. It would provide the tester with extra time so that more cows could be tested in one day, or more time could be devoted to conferences with the dairy farmer.
4. It would allow the dairy farmer to understand readily the method used in estimating production records, and to make the calculations himself if necessary.
5. It would save much time in the recording and checking of production data which are to be used for research purposes.

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THE STRUCTURE AND PROPERTIES OF THE NATURAL FAT GLOBULE "MEMBRANE"*

A HISTORICAL REVIEW WITH EXPERIMENTS BEARING ON A PHYSICO-CHEMICAL EXPLANATION

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HISTORICAL

Babcock (2) probably was the first American chemist to investigate emulsion character of milk and cream and to reject Ascherson's (1) haptogen membrane theory which had already prevailed for 45 years.

According to Babcock (2), in 1885 the fat globules were considered to be either (a) "particles of free fat, in the form of an emulsion with the serum of the milk," or (b) "surrounded by a thin membrane, and therefore cells filled with fat," or it was believed (c) "that the albuminous matter of the milk is attracted and in some way condensed upon their surface, forming what is called *haptogenic* membrane."

It is not clear why Babcock distinguished between the "cells filled with fat" and the haptogen membrane theory of Ascherson (1) (whom Babcock did not mention by name) since Ascherson's paper clearly considers capillary condensation of albumin and aggregation of an infinite number of small particles at fat surfaces to produce "haptogen" membrane as identical descriptions of the property of "hymenogeny" which he discovered. Furthermore, Ascherson not only postulated that the fat globules in milk are surrounded by a "haptogen" membrane but he claimed to have seen the membrane by microscopic observation, both in natural milk and in artificial emulsions of olive oil in dilute egg albumin solution.

Babcock disagreed with those who claimed to have seen the membrane both intact and after rupture as the "broken sacks" of the fat globules by suggesting a possible "lack of skill in the use of the microscope" as well as "influence of a preconceived opinion." It is obvious that Babcock himself favored the emulsion theory to account for the fat globules. An emulsion, he stated, is produced by dispersing liquid fat in an aqueous fluid having viscous properties whereby the fat particles are prevented from uniting again "by a thin film of liquid analogous to that which separates the bubbles of air in foam or soap suds." Babcock then pointed out the analogies between milk and artificial emulsions, (a) in microscopic appearance.

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(b) in creaming and the accentuation of the same by dilution with water, (c) on churning, especially the relation thereto and importance of temperature and melting point of fat, and (d) on their behavior when treated with ether. As a convincing argument Babcock pointed out that churns which had formerly been designed with the view of rupturing the haptogenic and other alleged membranes surrounding the fat globules were being replaced with those which would accentuate aggregation and coalescence of the fat globules into granules and of the granules into butter.

Babcock was soon led to modify his views somewhat regarding fat globule "membranes" due to his conclusion that traces (Babcock estimated 0.0002 per cent) of fibrin normally form in milk. In 1889 (3, 4) Babcock presented the evidence for the formation of "lacto-fibrin" and discussed the importance of this phenomenon at considerable length. While neither the evidence nor the alleged practical applications of the phenomenon are any longer valid, it is of interest that Babcock sought by means of his conclusions to explain the natural agglutination of the fat globules,¹ the relation thereto of gravity and centrifugal creaming and its influence on churning. Babcock believed that in cream the fibrin "clots have practically the same effect as would a true membrane covering the globules, and must be removed before the globules can unite in the form of butter." The acid of ripened cream was regarded as an effective solvent for this purpose. At the present time students who are being introduced to current theories in dairy chemistry are usually surprised to learn that while Babcock was one of the first to call attention to the natural fat globule clustering phenomenon, and later (5) showed its importance in explaining changes in the "consistency" (viscosity) of milk, he nevertheless regarded fat clustering as detrimental to the creaming and described methods for preventing it.

Current theories about the emulsions which occur naturally in biological material permit the acceptance of aggregates of oriented colloidal particles at the surface of milk fat globules to form "membranes" such as were not visualized by Babcock. Some of Ascherson's views, however, were remarkably prophetic of later discoveries.

The fact that milk plasma contains a number of proteins each capable of effective stabilization of fat emulsions has been the cause of much of the confusion in the literature as to whether cows' milk possesses a special fat emulsifying system. For a number of years I have employed the term membrane in quotation marks to describe this system. A major part of this literature, both old and more recent, may be found in the following publications (6, 16, 17b, 18, 20, 25, 30, 31).

Although Babcock (2) recognized that the milk emulsion is not destabilized by dilution with water, the Danish chemist Storch (24) was the first to

¹ The occurrence of a true agglutinin in milk which is adsorbed by the fat globules at low temperatures, causing their agglutination, is supported by the paper of Sharp and Krukovsky (JOUR. DAIRY SCI., 22: 743-752, 1939) in which the older literature is reviewed.

apply this fact to the problem of isolating the natural emulsifying agents through the process now commonly called cream washing. Separator cream is diluted with distilled water at approximately body temperature (Storch used both water and sucrose solution) to give a fat content similar to whole milk and the diluted cream re-separated. If one starts with fresh cream from the freshest possible milk this process may be repeated until the tests of the washings for milk plasma constituents are essentially negative without impairing the stability of the emulsion. Surface and interfacial adsorption equilibria are, of course, sensitive to temperature changes. If the cream washing operation is conducted at animal temperature it is reasonable to conclude that the fat globules of such washed cream are "coated" by the emulsifying agents present when the milk is secreted together with other substances whose presence could be explained by chemical or physical affinity with the fat or its emulsifying agents. With these hypotheses as a background, it becomes largely a chemical problem to isolate and identify the natural "membrane" components.

Storch's (24) experiments pointed the way to an important aid in the isolation of the "membrane," which I and my associates (16) developed and employed extensively in our later studies (17a, 18, 20, 25, 30, 31), namely, the release of a large part of the protective agents into the buttermilk during the churning of washed cream whereby both the free buttermilk and that released by melting the butter become important source materials for chemical studies. Indeed, not only was it found (20, 29) that the emulsion properties and churnability of artificial emulsions of milk fat in the various colloidal sols from milk plasma are strikingly different from those of washed natural cream but also that the "membrane" materials isolated from the washed artificial creams are also chemically distinct from the natural "membrane" substances.

Only a relatively brief account can be given of the discovery of the specific components of the natural "membrane." Storch (11) first postulated a specific protein but its supposed mucoid nature was not substantiated. The protein is salted out of aqueous sols like a globulin (16) but does not require electrolytes for dispersion (18). As isolated by Hattori (10), by Samuelsson (16) and by Wiese (18), by Rimpila (20), and by Tarassuk (17b), and by Schwarz and Fischer (22), the protein is characterized by a N content several per cent lower than that of any other milk protein, a fact not yet explained either on the basis of known amino acid composition or identified prosthetic groups (30, 10, 22). The biological specificity of the protein was established by Lewis (15).

Dornie and Daire (8) postulated that the higher lecithin content of buttermilk than of whole milk arose from the release of lecithin from Storch's fat globule "membrane" but Samuelsson (16) first supplied direct evidence in support of this hypothesis by isolating phospholipides from washed cream

buttermilk. Wiese (18) later identified lecithin, cephalin and sphingomyelin-like phosphatides in the natural "membrane" material. Thus, the specific emulsion stabilizing agent of cow's milk was finally established as a protein-phospholipide complex. Its aqueous sol was found (18) to have an isoelectric point at pH 3.9-4.0. The importance of the phospholipides in the hydrophilic properties of the membrane has been emphasized by Pyenson and Dable (19). Sandelin (21) believes that lecithin is the more important component of the membrane in explaining the stability of the milk and cream emulsion.

A third major lipid component of the isolated natural "membrane" was encountered by Wiese (18) and Rimpila (20) in the form of a neutral, high melting glyceride, the significance of which is still obscure.

The more important components of the natural "membrane" present in minor quantity are enzymes and heavy metals. Toyama (26) first showed that the crude "membrane" serves as a satisfactory concentrate of xanthine oxidase, thus confirming the view of Wieland and Macrae (29) that cows' milk dehydrogenase is closely associated with the fat globules. Sharp (23) states that about one-half of the xanthine oxidase may be removed from the fat globules by washing. He also reports that milk contains about 70 milligrams of the enzyme per 100 grams of fat. This would constitute 12-15 per cent of the protein of the membrane, based on Rimpila's (20) data regarding the protein:fat relationship of washed cream. Xanthine oxidase being a riboflavin-protein compound accounts for the fact that both raw sweet cream buttermilk and the buttermilk from churning washed cream have a brownish-yellow color. Kay and Graham (13) first demonstrated that phosphatase is concentrated on the fat surfaces of milk. Rimpila (20) found that 50 per cent of the Kay-Graham phosphatase of fresh cream remains after six washings, each with four volumes of water. Davies (7) found that the "membrane" protein readily combines with copper and iron which dissolves in milk and cream in milk processing plants, thus causing a concentration of these metals on the surface of the fat globules. The probable importance of metallic ions, especially of copper ions that might arise from these compounds, in contributing to the oxidative deterioration of cream and butter, may readily be conjectured.

EXPERIMENTAL

The character of the specific components of the natural fat globule "membrane" of cows' milk so far identified raises both physiological and physico-chemical questions regarding their origin. The supposition that they are artifacts of the cream washing procedure is not rational. Physiological explanations of their origin are still in the realm of speculation but it is difficult to ignore the thought that there is some intimate relationship between them and the synthesis and secretion of milk fat. The physico-

chemical aspects of the question are capable of experimental approach. Milk plasma contains colloidal systems which are excellent emulsion stabilizers. If it could be determined that the specific components of the natural membrane are preferentially adsorbed by a milk fat surface because of normal differences in their interfacial tension reducing ability as compared with the plasma colloidal systems, the origin of the natural "membrane" would have a physico-chemical explanation which could be regarded as at least plausible.

Dr. M. E. Powell carried out an extensive study of this question in my laboratory during 1932-34.² None of the results have heretofore been pub-

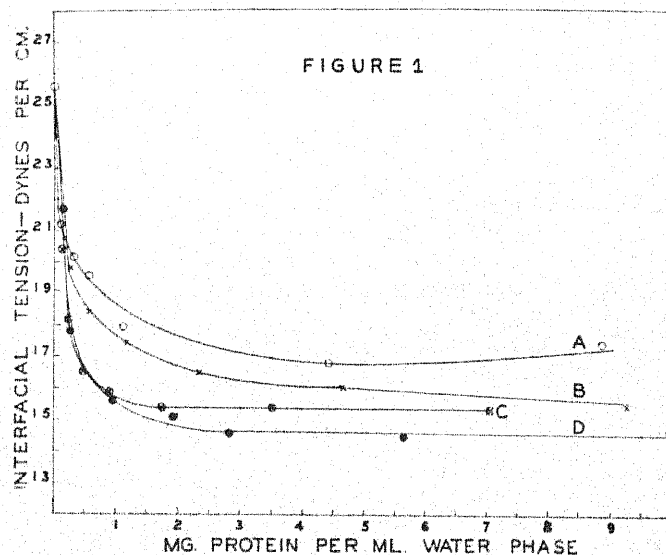


FIG. 1. Comparative tension reducing ability at water-butter oil interface of proteins (1) in lactalbumin sol A; (2) mixed milk serum protein sol B; (3) milk plasma sol C; and (4) calcium phosphocaseinate sol D, pH 6.7. Readings at 40° C.

lished. Using the drop weight method of Harkins and Brown (9) the interfacial tension reducing ability of different concentrations of the various colloidal systems of milk, including the natural "membrane" system, was determined at a butterfat-water interface at 40° C. This made it possible to determine at what concentration of protein a minimum interfacial tension was attainable for each system and gave a comparison both of the absolute ability of each material to reduce the interfacial tension of the butterfat-water interface and of the relative effectiveness of each to produce its own minimum tension.

In this work we observed for the first time that freshly washed fresh cream is sufficiently stable to withstand homogenization at 3000 lbs. pres-

² This work was supported by a grant from the Rockefeller Foundation.

sure at 105° C. This suggests that the materials remaining at the fat surfaces of washed cream represent either multimolecular layers or monomolecular complexes capable of considerable distensibility without impairment of the stability of the emulsion. The capacity of the resulting interface to adsorb the various colloidal capillary active systems of milk was examined experimentally in interfacial tension studies.

Figures 1, 2, 3, 4 and 5 present graphically some of the results of the various interfacial tension measurements.

Figure 1 shows the relative interfacial tension reducing ability of (a) lactalbumin sol (whey dialyzed against distilled water), (b) milk serum proteins sol (whey dialyzed with addition of NaCl), (c) milk plasma (skim

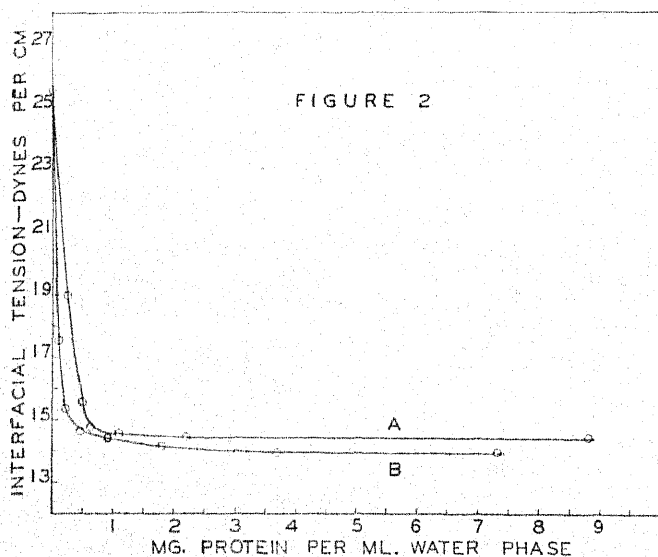


FIG. 2. Comparative tension reducing ability at water-butter oil interface of proteins in (1) milk plasma sol A; (2) sweet cream buttermilk B, from the same milk. Readings at 40° C.

milk) and (d) calcium phosphocaseinate sol (pH 6.7), each per unit of protein, their effectiveness being in the order named. Figure 2 shows the definitely greater effectiveness of sweet cream buttermilk over milk plasma proteins in reducing interfacial tension, both products being from the same original milk.

Figure 3 shows the markedly greater effectiveness, on the protein basis, of buttermilks from the melted butters of washed creams (curves A and B) than of the free buttermilks of the same washed creams (curves C and D) in reducing interfacial tension. These results seem to support the recent findings of Maimistova (14) that the capillary activity of the fat globule "membrane" is conditioned by its phospholipide compounds. We have, in

our laboratory, unpublished evidence that relatively greater proportions of phospholipide remain in the butter churned from washed cream than is liberated in the free buttermilk. The findings of Sandelin (21) also support this.

Figure 4 shows the effects on interfacial tension between butterfat and homogenized washed cream of additions to the latter of two samples of milk plasma (skim milk), curves A and B, a calcium phosphocaseinate sol, curve C, and a concentrate of free buttermilk from an unhomogenized portion of the same washed cream which had been homogenized, curve D. The rise

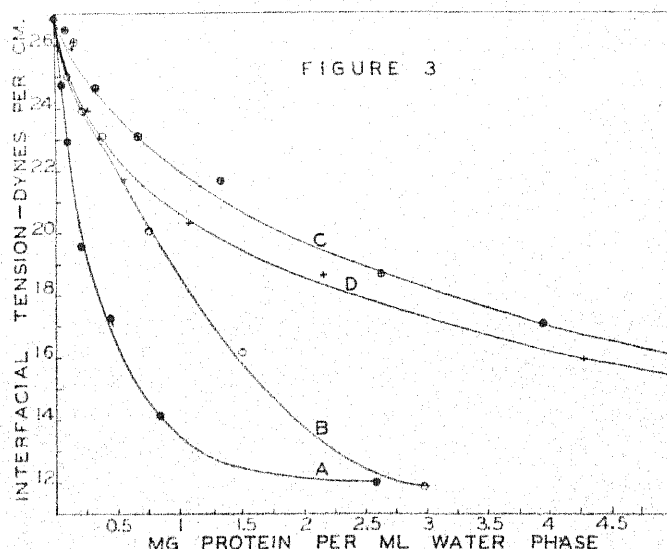


FIG. 3. Comparative tension reducing ability at water-butter oil interface of protein in (1) buttermilks from melted butter of washed creams A and B; (2) free buttermilks C and D, sols A and C being from the same washed cream, likewise B and D. Readings at 40° C.

in interfacial tension after the minimum had been reached in curves C and D suggests regions of concentration of the materials where less adsorption of capillary active colloids on the homogenized fat globule surfaces occurred. Why this should be so is not clear. Attention is called to the fact that figure 4 requires a different interpretation than figures 1, 2 and 3 since we are probably dealing here with colloids which remain free to reduce the tension between the butter oil and the water phase of the homogenized washed cream after the fat surfaces of the latter have attracted such material as can be adsorbed.

Figure 5 shows further evidence in support of the conclusions drawn from figure 3, that the phospholipide-protein complex retained by the butter of washed cream is more effective, on the protein basis, than the free butter-

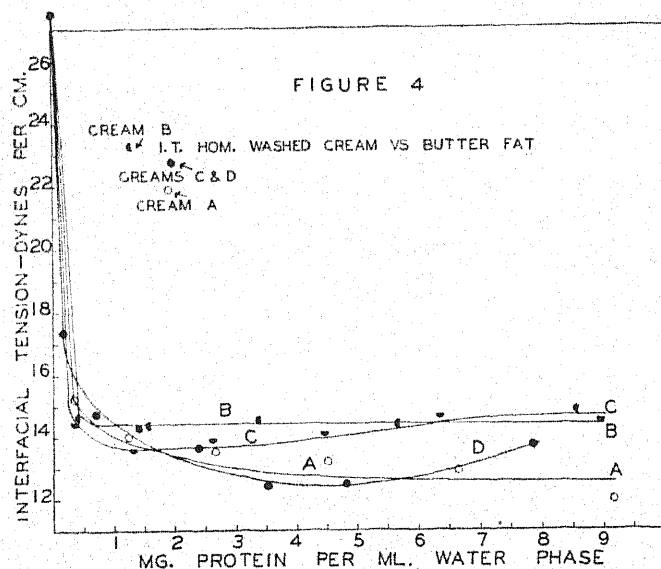


FIG. 4. Comparative tension reducing ability at homogenized washed cream-butter oil interface of proteins in (1) milk plasmas A and B; (2) a calcium phosphocaseinate sol C; and (3) a concentrate of free buttermilk D from an unhomogenized portion of the same washed cream which had been homogenized to furnish the interface. Readings at 40° C.

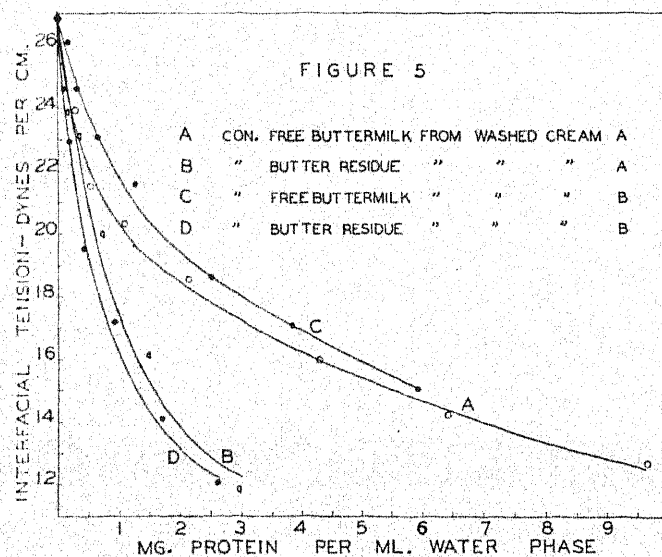


FIG. 5. Comparative tension reducing ability at water-butter oil interface of proteins in (1) concentrated free buttermilk A and concentrated aqueous phase of butter B, both from the same washed cream A; (2) concentrated free buttermilk C and concentrated aqueous phase of butter D, both from same washed cream B. Readings at 40° C.

milk from the same washed cream in reducing the tension at a melted butter-fat-water interface.

General importance of "membrane" in dairy technology. The demonstrated fact that the fat phase of cows' milk is stabilized by a complex (or complexes) of the lecitho-protein type, involving a specific protein and having associated with it concentrations of redox and phosphatase enzymes, suggests numerous problems involving their probable importance in dairy technology. Both Wiese (18) and Rimpila (20) found certain variations in composition of the natural "membrane" which are no doubt of considerable significance. The relation of these facts to various theories of churning has been discussed by Palmer and Wiese (18). It cannot be questioned that there is considerable release of natural "membrane" during the churning and theories supposing the necessity of foaming and of coagulation of the "membrane" protein are made untenable.

Thurston and Barnhart (27) found important relations to exist between "richness" of flavor in milk products and the phospholipide fractions of the "membrane." Thurston and associates (28) presented evidence supporting their belief that certain off flavors of milk, particularly oxidized flavor, are associated primarily with chemical deterioration of the lecithin of the fat globule "membrane" rather than with oxidation of the butterfat itself. Jack and Dahle (11) have presented evidence suggesting the probability of a double layer membrane on the surface of the fat globules, the outer layer of which must be removed in order to secure centrifuged cream of highest fat content. That the natural "membrane" material released during churning may explain, in part at least, the low curd tension of sweet cream buttermilk is indicated by experiments carried out in the author's laboratory (17a). Many of the normal properties of natural cream have been found to require the natural "membrane." This is true for centrifugal cream separation, gravity creaming and churning (30) and for desirable whipping properties of ice cream mixes (12). For the latter the significant aspect is the protein-phospholipide complex which is thus capable of being imitated by other natural complexes of this type, *e.g.*, by that in egg yolk.

SUMMARY

The fat globules in cows' milk are wholly or partially surrounded by a special group of substances whose origin may be due, in part, to their greater capillary activity. The other surface active substances occurring in major concentration in milk plasma evidently constitute the outer layers of the fat globule surfaces if indeed they are normally concentrated there at all. The latter are readily removed when cream is washed by dilution with water. Experimental work in the author's laboratory and by numerous other workers cited, has pointed to the importance of the natural "membrane" of the fat globules in creaming, churning, milk flavor (both normal and oxidized),

decreased curd tension of natural sweet cream buttermilk, and in determining the desirable whipping qualities of ice cream mixes.

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IMPROVING THE QUALITY OF SWISS CHEESE BY CLARIFICATION OF THE MILK¹

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It is commonly recognized that good quality in the milk is an important factor in the production of high quality cheese. For making Swiss cheese the suitability of any milk appears to be enhanced by the agitation or centrifugal treatment given milk by clarification. Orla-Jensen (20) was the first to observe that centrifuging milk resulted in improvement in Emmentaler (Swiss) cheese by the formation of fewer and larger eyes, and that a similar effect resulted when the cheese milk was filtered and likewise when it was agitated more than usual while being transported for a long distance. This process for improving Swiss cheese was first described in this country by Matheson in a preliminary report (16) and in a public service patent (17) from these laboratories. Its use has resulted in such marked improvement in eye formation and in the quality of the cheese generally that it has been adopted in practically all Swiss cheese factories in this country. The most obvious effect on the quality of the cheese is an increase in the size of the eyes and a decrease in their number, this resulting in a distinct improvement in grade and market value. The effects on the cheese appear to be caused by alterations in the composition of the milk and in the physical condition of the constituents of the milk, both of which factors result in conditions conducive to bacterial action of a type which favors the development of the proper texture in the curd and desirable eye formation.

Previous to 1924 little was known as to the mode of action of clarification in improving the quality of the cheese. It was thought to come about through "removal of dirt or other cellular elements from the milk" (17), and it was mentioned also (16) that the process "breaks up the clusters of fat globules." Orla-Jensen (20) believed that the improvement resulted from the effects of agitation in distributing uniformly gas-forming organisms, especially those associated with particles of foreign material. More recently, Guittonneau and his associates (6) indicated that particles of for-

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¹ The cheesemaking phases of the investigations were directed by the late Kenneth J. Matheson, who was largely responsible for improvement in quality of domestic Swiss cheese by clarification. The introduction in the factories of the clarifying process, and also of the pure culture method, was accomplished largely by other former employees, including Sumner A. Hall, Robert E. Hardell, James A. Boyer, Robert R. Farrar, and Fred Feutz, and by H. R. Loehry of this Division.

² Deceased April 24, 1940.

eign material, when deposited in the cheese, act as centers of proliferation of undesirable gas-forming organisms, and that the improvement in eye formation results from the removal of large numbers of the organisms that occur as contaminants in and on the heavier particles of sediment.

Some of the favorable effects reported as due to clarification of milk on cheese include increased firmness of body of Swiss cheese (16), improved body (3, 5, 34) and flavor (3, 5, 22, 34) of Cheddar cheese and the prevention of gassiness, pinholes, and sponginess (1) in the curd of Cheddar cheese.

Reports of the effect of clarification on the milk in the removal of extraneous matter and cellular material have shown that the clarifier removes practically all visible sediment (10, 13, 19), that a portion of the fibrin (common in mastitis milk) is removed (13), that from about two-fifths to about two-thirds of the leucocytes and body cells are removed (4, 7, 10) and many of those remaining in the milk are fragmented (15), and that the proportion of cells removed increases as the temperature of clarification is increased (10). Some investigations have shown that the larger types of organisms are removed in greater proportion than the smaller ones (13), and also that, when the rate of flow of milk through the bowl is reduced, there is some selective removal of different types of bacteria, causing actual decreases in plate counts (34). On the other hand, the numbers of streptococci in the milk are not reduced significantly (28) and investigators (4, 7, 10, 13, 34) have agreed that clarification results in an increase in the total number of bacteria in the milk as determined by the plate count, due to breaking up of chains and clumps of bacteria. This change in the bacterial flora by clarification does not improve the keeping quality of the milk, since it has been shown that the methylene blue reduction time is often decreased (10, 28) and that there is a slight increase in the rate at which acidity develops (7, 19, 28).

It has been stated that clarification results in a reduction in size and an increase in the number of fat globules (19, 4) in milk and breaks up fat clumps (4). It is well known that, at temperatures used commonly, the process causes a decrease in the rate of creaming and a decrease in the volume of gravity cream (4, 7, 10, 14, 19, 33); at relatively high temperatures it causes an additional decrease in the volume of gravity cream (4, 10, 33). A reduction in the volume of gravity cream is produced also by pumping milk with a centrifugal pump (33), and by mechanical agitation (30). The latter treatment was found to break up clusters of fat globules. Agitation is believed to alter the surface characteristics and adsorption on the fat globules (11), with which the creaming property is associated (21); the fat-clustering agent, agglutinin, believed to be present in milk, is thought to be removed to some extent by agitation and centrifugal force (8); and the agglutinating material, when adsorbed on the surfaces of solid fat globules, can be released rather readily (27) by such a mild treatment as raising the

temperature to above that of the melting point of the fat. Further indication that clarification of milk alters the nature of the fat is provided by evidence that, in the manufacture of dried milk, clarification of the milk increases the resistance of the fat to oxidative decomposition and improves the keeping quality (9).

The alteration in the gaseous content of milk resulting from the centrifugal agitation in the clarifying process may be considered as another desirable factor attributable to clarification in the manufacture of cheese. Babcock (1) showed that the centrifuging of milk tended to prevent gassiness in Cheddar cheese curd and he believed this effect was due to aeration, which presumably alters conditions for bacterial growth and inactivates certain types of gas-forming bacteria. Marshall (12) found that agitation of milk promoted an interchange of gases, thus increasing the ratio of oxygen to carbon dioxide, and that such an interchange inhibited undesirable fermentations. Results of Matheson (16) showed that the addition of ozone or of oxygen to milk inhibited the gassy fermentation produced by a vigorous, gas-producing, spore-forming anaerobe in Swiss cheese, without apparent injurious effect on eye formation or on development of the lactobacilli in the cheese.

It is obvious that clarification effects removal of the extraneous matter and also alters the physical properties of some of the constituents of milk. The evidence available indicates also that the changes produced by clarification result in conditions more favorable for the growth of the types of organisms that are useful in the cheesemaking process. From the wide variations that appear in the quality of cheese made commercially from clarified milk, however, it appears that its effects are more pronounced in milks possessing certain abnormalities, and that in some cases the milk is not clarified properly. This paper is a report of experiments designed to contribute additional information on the effects of clarification on the properties of milk; to study the effects of certain related treatments of milk which influence the quality of Swiss cheese; and to verify in a quantitative way the effects of clarification on the quality of the cheese. During part of this investigation there was available a supply of milk selected from cows having mastitis, and the results of clarification studies on this milk are included because of the unusually pronounced improvement that occurred when abnormal milk of this type was clarified.

METHODS

In efforts to account for variations in the quality of the cheese, those properties of milk which appeared to be of possible significance were determined in samples of the mixed milk taken from the cheese kettles. Counts of clusters of fat globules and of numbers of globules per cluster were made, at a temperature of 25° to 26° C., by means of a microscope in milk samples

diluted 1:24 in an aqueous solution containing 1.5 parts of gelatin and 1 part of phenol per 100 parts. The phenol was used as a preservative in the gelatin solution after it was determined that its presence in the test did not affect the tendency of the fat globules to form clusters. Pairs of samples were placed in a calibrated Levy counting chamber (hemacytometer) with double ruling and allowed to remain for one to one and one-fourth hours for clustering to occur before counts were made. At least 50 fields per sample were examined. Groups containing 10 or more globules together were counted as clusters. For measuring sizes of fat globules an ordinary cover glass was used on the counting chamber and measurements were begun at once without allowing time for clustering to occur. A calibrated ocular micrometer disc, and also an ocular disc with circle and cross lines, were used in measuring and counting.

The creaming ability of milk was measured in samples held for 24 hours in 100-ml. graduated cylinders of uniform height immersed in a water bath at 3° to 5° C. (graduated cylinder method).

The creaming ability of milk was measured also by a centrifugal procedure designed to shorten the time required for the completion of creaming and designated as the cream test bottle method. Large-bodied, 50 per cent, nine-gram cream test bottles were selected which, when filled to the 50 per cent mark, contained not less than 55.2 ml. Two 17.6-ml. samples of milk, a total of 35.2 ml., were pipetted into each bottle and 20 ml. of water was added. The sample was mixed thoroughly and then centrifuged for one hour at room temperature in a Babcock centrifuge, after which the volume of cream was read with the aid of a light placed behind the bottle. Since each whole percentage division represents a volume of 0.1 ml., the percentage of cream is calculated by multiplying the whole percentage unit volume of cream by 0.1, dividing by the volume of milk used (35.2 ml.), and multiplying by 100.

Methods described in an earlier publication (25) were used for determining rennet curd tension, rennet coagulation time, and stability to alcohol. Determinations of pH values were made by the quinhydrone electrode method described earlier (23). Amounts of oxygen and of other gases were determined by means of the Van Slyke (31) manometric blood gas apparatus. The numbers of organisms in the kettle milks were determined by the standard plating method in tomato-milk powder agar, and the numbers in cheese were determined microscopically (2). The counts of mastitis streptococci were made in Edwards' blood agar medium.

In the cheese experiments, cheese was made in pairs from two weighed portions of the same lot of mixed milk, and each test cheese was made in the same manner as the control except for the experimental variations described. The clarifier used was of the old style and studies with the new no-foam clarifier were not made. The usual clarifying temperature was 28–30° C.

The internal diameter of the clarifier bowl was 204 mm. (8 inches), the capacity 4,000 pounds per hour, and the rated speed 7085 rpm. Each experimental cheese weighed 55–62 pounds when removed from the press. Curing conditions and details of grading were the same as those described earlier (24).

The starter culture used in the earlier experiments was 39a (now identified as *Lactobacillus lactis*). In later experiments, either B₂ (a culture of *Lactobacillus bulgaricus*) or Ga (a mixed culture of a lactobacillus with a mycoderma) was used. While the Ga culture has been used extensively in the manufacture of Swiss cheese in this country for a number of years, studies of the lactobacillus in this culture have not as yet shown it to be identical with any species of lactobacillus described in the literature. The later experiments included the use of *Streptococcus thermophilus* cultures in addition to the lactobacillus cultures referred to above.

A penetrometer was used for determining softness of body of cured cheese. It consisted of a cylindrical plunger one-eighth inch in diameter, with a flat end, mounted on a frame, surmounted by a 200-gram weight, and connected to a needle which moved on a dial graduated to show the movement of the plunger in hundredths of a centimeter. The distance that the plunger sank into a small block of cheese in 15 seconds was recorded, and the average of five determinations was taken as the penetrometer reading. Determinations were made at a constant temperature of 18° C., and also usually at two different temperatures so that changes in softness with temperature could be plotted.

EFFECTS ON PROPERTIES OF MILK

The average volume percentages of cream obtained on 12 pairs of samples of milk were as follows: Graduated cylinder method—clarified milk, 10.2; unclarified milk, 10.9; cream test bottle method—clarified milk, 9.8; unclarified milk, 10.9.

Measurements of the sizes of the fat globules determined microscopically in a large number of samples showed that the decrease in creaming could not be accounted for on the basis of a diminution of sizes of the globules. There was a slight increase following clarification in numbers of those more than 4 μ in diameter and a more evident increase of those more than 6 μ in diameter. Some relatively large globules (larger than 10 μ) were found much more frequently in clarified than in unclarified milk, and some of the largest globules were found to have apparently coalesced during clarification. The resulting clumps were in some cases non-spherical in shape, and this effect was greater in milks clarified at 32° C. than in those clarified at 21° C.

It was found that the tendency of the fat globules to aggregate in clusters during creaming was reduced greatly by clarification. Data show-

ing the effect of clarification on clustering and the effects of different modifications of the clarifying process on the numbers of clusters are shown in table 1. Counts in a large number of samples showed also an average reduction of about 20 per cent in the numbers of globules per cluster following clarification.

When cream that had been allowed to rise by gravity was clarified and remixed with the original skim milk it was found that the tendency of the fat to aggregate in the milk was reduced. A similar effect on clustering was produced when the gravity cream was agitated for 10 minutes at 40° C. and then remixed with the original skim milk.

TABLE 1

Effects of clarification and of other treatments of milk on average numbers of fat clusters in milk and on average extent of over-setting defect in Swiss cheese

Treatment of milk	Pairs of samples	Fat clusters per 0.01 cu. mm. milk		Eyes per cut surface of cheese	
		Number	Decrease in number	Number	Decrease in number
	<i>number</i>		<i>per cent</i>		<i>per cent</i>
Not clarified	30	186	106
Clarified	73	61	54	49
Clarifier speed 3500 rpm.	25	156	77
Clarifier speed 7000 rpm.	72	54	59	23
Clarified at 21° C.	26	134	73
Clarified at 32° C.	86	36	64	12
Gravity cream not clarified	16	173	98
Gravity cream clarified	92	47	65	34
Gravity cream untreated	10	172	118
Gravity cream heated and agitated	66	62	103	13

Results of the alcohol test showed that stability of milk proteins to alcohol is decreased slightly by clarification, indicating that the process alters the properties of the casein slightly.

The rennet coagulation time of milk was found to be unchanged following clarification. The rennet curd tension was not altered significantly. The pH value was usually unchanged, but in some instances was reduced slightly. The methylene blue reduction time at 37° C. was usually not changed materially but in some instances was decreased slightly.

In experiments in which 18 lots of mastitis milk were made into cheese (table 3), the average of the numbers of leucocytes was decreased by clarification from 2.4 millions to 0.7 million per milliliter, or about 70 per cent. The numbers of streptococci and of other bacteria were decreased also, but with less consistency and to a less extent. The percentage reduction in numbers of leucocytes tended to be greatest in those milks containing the

largest numbers. The reduction varied directly with the speed of the clarifier bowl and with the temperature at which the milk was clarified, and inversely with the rate of flow of the milk through the clarifier.

Effects of clarification and of the addition of oxygen and of carbon dioxide upon the gas content of milk are shown in table 2. Clarification increased the oxygen content, decreased the carbon dioxide content, and decreased the total gas content. It will be noted that the interchange of gases resulting from clarification is in the same direction as that produced by the artificial introduction of oxygen, which has been shown by Marshall

TABLE 2

Effects of clarification, and of treatment with gases, on the percentages of gases in milk

Treatment of milk	Pairs of samples	Duration of gas treatment	Average amounts of gases in milk			
			Oxygen	Carbon dioxide	Nitrogen and residual gases	Total
	<i>number</i>	<i>minutes</i>	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>volumes per cent</i>	<i>volumes per cent</i>
Clarified	14	0.60	1.75	1.20	3.55
Not clarified	0.55	2.10	1.20	3.85
Oxygen added*	13	10	0.69	1.84	1.29	3.82
Normal	0.59	1.93	1.37	3.89
Carbon dioxide added*	7	5	0.52	3.58	1.22	5.32
Normal	0.58	1.84	1.24	3.66

* Gas bubbled through small openings in a perforated coil, at rate of 25 liters per minute, into 700 lb. milk in cheese kettle; temperature of milk, 28-30° C.; samples analyzed immediately after treatment.

(12) and by us (16, 18) to have a favorable influence on the activities of desirable types of organisms in milk.

Direct counts of Swiss cheese starter organisms made microscopically on five pairs of inoculated samples of milk that were subjected to the same temperature conditions existing in the cheesemaking process showed that the growth of the lactobacilli began considerably earlier in clarified than in unclarified milk and that the growth of both the lactobacilli and the streptococci progressed more rapidly in the former than in the latter. It was found also that, in milk samples held at 30° C., clarification caused the oxidation-reduction potential to decrease more rapidly.

EFFECTS ON QUALITY AND PROPERTIES OF CHEESE

Data for 369 pairs of cheese, showing the improvement of quality resulting from clarification and also the effects of various modifications of the clarifying process, are presented in table 3. In the cheese made from normal milk by the usual clarifying process (group 1), practically all of the im-

TABLE 3
Improvement in quality of Swiss cheese by clarification of milk, and effects of modifications of the clarifying process. (Averages for 60-lb. cheese cured 2½ to 3½ months)

Treatment of milk	No. of pairs	Scores of cheese*			Proportion of cheese in each grade			
		Eyes	Body and texture	Total	No. 1	Special	No. 2	Grinder
		points	points	points	%	%	%	%
1) Normal milk, clarified	112	25.4	23.0	75.2	49.1	34.8	9.8	6.3
Normal milk, not clarified		18.7	23.8	69.0	11.6	37.5	40.2	10.7
2) Mastitis milk, clarified	18	22.4	24.0	73.5	22.2	61.1	16.7
Mastitis milk, not clarified		12.2	23.2	61.2	5.6	11.1	22.2	61.1
3) Clarified	4	25.2	23.0	74.7	25.0	50.0	25.0
Separated		23.0	21.5	71.2	50.0	25.0	25.0
4) Clarifier speed—7000 rpm.	65	22.0	23.0	72.0	30.8	32.3	24.6	12.3
Clarifier speed—3500 rpm.		21.0	23.5	70.0	24.6	24.6	36.9	13.9
5) Clarified at 32° C.	63	23.8	22.7	73.2	44.5	23.8	23.8	7.9
Clarified at 21° C.		21.6	22.4	70.5	28.6	22.2	34.9	14.3
6) Normal rate of flow of milk through clarifier	16	24.2	22.4	73.6	31.3	37.5	25.0	6.2
Slow flow, half normal rate		25.2	23.8	75.6	50.0	31.3	12.5	6.2
7) Sediment from bowl returned to clarified milk	54	23.1	24.0	73.4	44.4	31.4	13.0	11.2
Milk clarified, sediment not returned to milk		23.2	22.0	71.7	29.6	29.6	29.6	11.2
8) 90-lb. gravity cream not clarified, 600-lb. gravity skim-milk clarified	36	17.6	23.1	67.7	5.5	36.1	27.8	30.6
90-lb. gravity skim-milk not clarified, rest of milk clarified		22.3	22.7	71.6	38.9	19.4	27.8	13.9
9) 90-lb. gravity cream clarified, 600-lb. gravity skimmilk not clarified	1	23.0	25.0	78.0	100.0
90-lb. gravity skim-milk clarified, cream and rest of milk not clarified		18.0	24.0	72.0	100.0

* Perfect score in points, according to scorecard used: eyes, 40; body and texture, 30; flavor, 20; and appearance, 10.

provement was in eye formation, *i.e.*, the eyes were larger, less numerous (less of the "oversetting" defect), shinier, and more regular and uniform in shape and distribution. There was a slight but uniform tendency toward more of the glaesler (curd-splitting) defect, and also more firmness of body, in the clarified than in the unclarified milk cheese. The former were generally slightly lighter in color and tended to rise somewhat more slowly in curing in the warm room. There were scarcely ever any detectable differences in flavor. Effects of different variations of the clarifying process upon the extent of the oversetting defect in the cheese are shown in table 1. The results show a striking correlation between the reduction in the fat clustering tendency in the milk, as affected by clarification, and the reduction in the oversetting defect in the cheese.

The greatest improvement resulting from clarification occurred when mastitis milk was used (group 2). Cheese made from this milk, without clarification, was wholly or partially pin-eyed (either pressler or nissler) in 15 instances out of 18. There were no pin-eyed cheese among those made from clarified milk. The unclarified, mastitis milk cheese was very soft or weak in body, relatively high in moisture content, and usually slightly inferior in flavor. Clarification caused an increase in firmness of body and a decrease in moisture content—factors which improve the quality of soft-bodied cheese.

Statistical tabulations of data for 110 wheels made from milks in which the leucocyte counts varied between 250,000 and 4,000,000 per milliliter showed a definite relationship between the numbers of leucocytes in the milks and the incidence and extent of the oversetting defect in the cheese. There was a strong tendency for high leucocyte counts in milk to be associated with "slow-working" or so-called "dead" milk in the kettle, with relatively slow development of acidity in cheese on the press, and with weakness of body in the cheese. These conditions are indicative of retardation of activity of starters and insufficient drainage of cheese on the press. They were improved markedly by clarification.

The use of the separator bowl did not produce as much improvement as the use of the clarifier bowl (group 3). In four experiments in which the milk was clarified twice, no additional improvement resulted from the second clarification. The improvement in quality was diminished when the speed of the bowl was decreased (group 4) and also when the milk was clarified at a relatively low temperature (group 5). The quality was improved when, with the bowl running at full speed, the rate of flow of milk through the bowl was diminished one-half (group 6). Results with the clarifier indicated that the improvement was generally proportional to the agitation or force to which the milk was subjected.

It is commonly believed that the improvement resulting from clarification is caused largely by the removal of extraneous matter or visible dirt.

However, the average quality of cheese made from clarified milk was found to be improved slightly, with respect only to the body, when the sediment from the bowl was returned to the clarified milk and mixed in thoroughly (group 7). Moreover, filtering the milk through cotton (12 pairs of cheese, unclarified milk) did not improve the quality of cheese to more than a very slight extent. Addition of the sediment did, however, cause a rather marked softening of the body of the cheese, which improved the body in this case because the cheese was ordinarily somewhat too firm; the average penetrometer reading on the cheese from clarified milk plus bowl sediment was 70; that on the normal controls, 45. The cheese to which the bowl sediment was added was also slightly more yellow and slightly less subject to the glaesler defect. The results indicated that improvement in eye formation cannot be ascribed to any great extent to the removal of visible sediment. It may be caused at least partially, however, by a change in the dispersion of the particles and by the effects of the process in breaking up bacterial clusters and distributing organisms more thoroughly in the milk, as Orla-Jensen (20) suggested.

Results of cheesemaking experiments with gravity cream (groups 8 and 9) indicated that effects of clarification on the fat in milk are apparently an important factor in improving the quality of cheese. When unclarified gravity cream (allowed to rise for 16 hours at 8–10° C.) was returned to the clarified milk (group 8), the quality of the cheese was so reduced as to make it comparable with that of cheese made from unclarified milk. When the gravity cream was clarified and then returned to unclarified gravity skim milk (group 9), improvement resulted which was comparable with that produced when all the milk was clarified. In further experiments made to determine effects of agitation on the fat, the gravity cream was warmed to 40° C., agitated vigorously for 10 minutes, and then re-mixed with the unclarified, gravity skim milk. This treatment resulted consistently in improvement in eye formation.

Prompted by results of Marshall (12) and of these laboratories (16, 18), referred to earlier, experiments were conducted to determine the effects of addition of oxygen and of carbon dioxide to milk. In 13 pairs of cheese the addition of oxygen (amounts shown in table 2) resulted in an average increase of six points in the scores of the test cheese. Twelve pairs were made with gaseous oxygen added to unclarified milk in the test kettle and with clarified milk in the control kettle. The average of the scores of the oxygen-treated, unclarified milk cheese was very nearly as high as that of the clarified milk cheese. Seven pairs were made from clarified milk with gaseous carbon dioxide added to the milk for the test cheese. The addition of carbon dioxide resulted in an average decrease of 10 points in the scores of the test cheese.

Results of counts of starter bacteria determined microscopically in samples taken from five pairs of cheese showed that the streptococci and

the lactobacilli, particularly the latter, multiplied more rapidly in cheese made from clarified than in that made from unclarified milk. A pronounced decrease in numbers of lactobacilli began after the cheese was one day old, and this decrease was more rapid in the unclarified-milk than in the clarified-milk cheese. The numbers of streptococci diminished somewhat later and more slowly than the numbers of lactobacilli, and they also diminished more rapidly in the unclarified-milk cheese. At three hours after dipping the average pH value in 112 pairs of cheese was 0.03 lower, and at eight hours 0.10 lower, in cheese made from clarified milk than in that made from unclarified milk. In nearly every instance cheese made from clarified milk contained less lactose when one day old than that made from unclarified milk.

There was a consistent increase in firmness of body of cheese as a result of clarification. Averages of penetrometer readings at 18° C. for 70 pairs

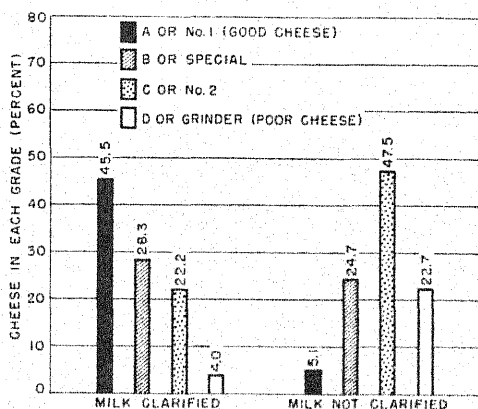


FIG. 1. Relationship between clarification of milk and quality of Swiss cheese (198 pairs of laboratory cheese).

of cured cheese made from normal milk were as follows: clarified, 35.1; unclarified, 55.0. Averages for 18 pairs made from mastitis milk were: clarified, 41.6; unclarified, 67.3. In many instances penetrometer readings were made at two or more temperatures and changes in softness were plotted against temperatures of readings. It was found that the clarified-milk cheese tended to soften less than the unclarified-milk cheese when the temperature was increased.

The glaesler (curd-splitting) defect occurred more commonly in cheese made from clarified than in that made from unclarified milk; the average difference in score for this defect was 0.5 point.

Averages of composition, yield, and fat loss data for 150 pairs of uncured cheese were as follows: clarified—moisture, 38.56 per cent; fat in dry matter, 47.7 per cent; yield, 9.65 per cent; fat in whey, 0.67 per cent; unclarified—

moisture, 39.19; fat in dry matter, 48.4; yield, 9.82; and fat in whey, 0.62. The decrease in yield resulting from clarification was apparently slightly greater than could be accounted for by the observed decrease in moisture content and the slight increase in fat loss in the whey. The lower moisture content in clarified milk cheese is undoubtedly of some importance in improving the quality, since it has been shown (24) that high moisture content is one of the factors responsible for inferior quality.

Data for 198 pairs of Swiss cheese, showing the average improvement in quality in our experiments, are presented in figure 1. A photograph illustrating the typical improvement in eye formation resulting from clarification of normal milk is presented in figure 2. The use of the clari-

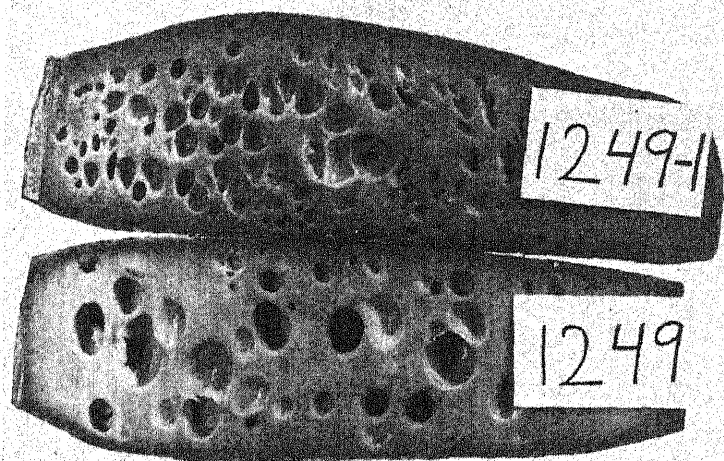


FIG. 2. Effect of clarification in improving the quality of Swiss cheese. No. 1249-1, milk not clarified. No. 1249, milk clarified.

fying process with normal milk increased the average grade from No. 2 to Special.

DISCUSSION

Although the removal of the extraneous matter is generally believed to be important in improving the quality of Swiss cheese, it was found in these experiments that the return of the bowl sediment to clarified milk did not injure significantly the eye formation of the cheese, although it tended to result in cheese of softer texture. The extent of leucocyte removal (about 70 per cent) by clarification was in agreement with the work of earlier investigators. The pronounced decrease in number of leucocytes, considered in conjunction with the fact that clarification resulted in an increase in the growth of the starter organisms in milk and in cheese, has suggested

the possibility that one factor responsible for relatively poor quality in unclarified-milk cheese made from milk having mastitic characteristics may be the inhibition or partial destruction of starter organisms by leucocytes. Whitehead and Cox (32) presented data which they believed indicated evidence of ability of leucocytes, particularly if present in large numbers, to retard the activity of lactic acid bacteria by phagocytic ingestion. In our work, however, microscopic examination of starter organisms grown in unsterilized, mastitis milk showed what appeared to be inconclusive evidence of phagocytosis of the lactobacilli by the leucocytes. The increase in acid development in cheese eight hours after dipping, resulting from clarification, indicates that some factor present in mastitis milk and partially removed or altered by the clarifier has a retarding effect on the growth of the lactobacilli.

The factor of agitation appears to be responsible for many of the changes resulting from clarification of the milk. Possibly, foremost among these changes is the dispersion and alteration of the fat. Results of other investigations, mentioned above, have shown that preliminary clustering of milk fat is essential to normal creaming. Our results indicate that the decrease in creaming in clarified milk is attributable primarily to a decrease in the clustering tendency. The evidence available agrees with the explanation of Hekma (8) that the change in the clustering tendency following clarification or agitation of milk results from a decrease in the amount of agglutinating material adsorbed on the surfaces of the fat globules.

Whether the improvement in the cheese following the clarification of the milk or the clarification or agitation of the gravity cream results in any degree from the reduction in the tendency of the fat to aggregate has not been determined. It is possible that when there are more large aggregates of fat, such as occur in unclarified milk, these clusters of fat may form "weak" areas in the cheese curd. Any such fat clusters containing foreign particles, which may be picked up from the milk and which are likely to contain unusually large numbers of gas-producing organisms (6), may serve as foci for abnormal eye formation in the cheese.

While the results of the experiments on the clarification and agitation of only the gravity cream indicated that the physical effect is largely on the fat, it cannot be concluded definitely that the beneficial effects are not also partially bacteriological—the breaking up and distributing of chains and clusters of organisms. Stine (29) has shown that when cream rises on milk the organisms tend to be carried upward with the fat and their numbers per milliliter in the cream layer may be 50 times as great as in the skim milk below. Schmidt (26) found an 85-fold proportion of *S. lactis* in the cream layer.

The results described above on the interchange of gases occurring during clarification and on the effects of adding oxygen and carbon dioxide to

cheese milk indicate that the effect of the process on the amounts of these gases in milk is an important factor in controlling the type of bacterial fermentation in a manner favorable to the proper ripening of the cheese.

The cheese made from clarified mastitis milk was of unusually high quality in view of the known disadvantages in the use of such abnormal milk for cheesemaking. It should be explained that the milk received regularly for this experimental work was from a herd having a rather large proportion of Jersey cows. The normal milk was therefore relatively high in solids content and the cheese made from it was relatively firm and of slightly poorer quality generally than would be expected from milk containing less solids. The effect of clarification on the experimental cheese resulted in greater improvement in the case of mastitis milk than in the case of normal milk.

The milk received in the factories, however, comes from herds which consist largely of Holsteins or of cows belonging to other breeds that produce milk of relatively low solids content. The cheese made in most factories is less firm and often tends to have a soft texture and even a weak body. The additional softening of the body resulting from mastitic characteristics of the milk is likely, on the basis of these results, to be particularly detrimental if the milk is not clarified.

SUMMARY

Experiments have demonstrated that clarification of milk produces a marked and consistent improvement in the quality of Swiss cheese. Studies were conducted of the properties of the cheese milk for the purpose of investigating the intermediate factors in the improvement in the cheese. Specific effects of clarification on milk include a decrease in the tendency of the fat to form aggregates upon standing, removal of a large proportion of the leucocytes from mastitis milk, an increase in the rate of multiplication of starter organisms and improvement in results of fermentation tests, an increase in concentration of oxygen and decrease of carbon dioxide, an increase in the rate at which the oxidation-reduction potential changes at 30° C., and a slight decrease in stability to alcohol.

Specific effects of the process on the properties of the cheese include a marked decrease in number and increase in size and uniformity of eyes, an increase in the firmness of the cheese and in the incidence of the glaesler defect, an increase in the rate of multiplication of starter organisms and of acid formation, a decrease in moisture content and in yield of cheese, and an increase in the fat loss in the whey.

The effects of clarification on properties of milk and on properties and quality of cheese were found to be diminished by clarifying the milk at a relatively low temperature and with a relatively slow bowl speed; they were increased by decreasing the rate of flow of milk through the bowl by

one-half, and by increasing the temperature from 21° to 32° C. The beneficial effects of clarification of milk for cheesemaking were especially pronounced in the case of mastitis milk.

Of the intermediate factors in the improvement of quality of clarified milk cheese, those that appear most significant are a decrease in aggregation of the fat globules, an increase in oxygen and decrease in carbon dioxide, improvement in effectiveness of starters, and a reduction in leucocytes when present in large numbers.

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PREVENTION OF MILKSTONE FORMATION IN A HIGH-TEMPERATURE-SHORT-TIME HEATER BY PREHEATING MILK, SKIM MILK AND WHEY

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In the course of experiments on high-temperature short-time forewarming of milk (6) it was observed that, when the milk was reheated, the pressure required to force the liquid through the heating, holding and cooling coils was nearly constant, whereas when raw milk was heated in this equipment more and more pressure was required to maintain a constant flow. This observation seemed of such practical value as to justify an investigation of the conditions under which the effect could be obtained, and an explanation of the cause.

EXPERIMENTAL

The equipment was, in general, the same as that described in a recent paper (6). The pump was the reciprocating type. It operated at 200 strokes per minute and had a capacity of 90 gallons per hour. The internal diameter of the stainless steel tubing through which the liquid was pumped at the uniform rate of flow of 22 feet per second was 0.18 inch. Three seconds were required to heat and 3 seconds to cool the liquid. When the holding time was 15 seconds, the total length of the tubing from the pump to the outlet at the end of the cooling coil was about 480 feet. The pressure required to pump water through this length of clean tubing at the rate of 90 gallons per hour was 2,000 pounds per square inch. The resistance to flow of more viscous liquids was, of course, greater. Under uniform conditions an operating pressure greater than that required when the tubing was clean was considered due to a coating of milk solids or the formation of "milkstone" on the inside wall of the tubing.

It is apparent that, under the above conditions, even a very thin deposit on this tubing would be reflected in the amount of force or pump pressure required to maintain a uniform flow of liquid.

When raw milk at room temperature was pumped through the system without heating there was no increase over the initial operating gage pressure.

Preheating a test liquid other than in the high-temperature equipment was done in steam-jacketed hotwells equipped with agitators. In one hotwell it was the practice to heat 40 gallons of liquid; in the other, 50 gallons. The period required to attain the desired temperature was approximately the

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same in each hotwell. To heat from 10° C. (50° F.) to 65° C. (149° F.) took 28 minutes; to 75° C. (167° F.), 33 minutes; to 85° C. (185° F.), 40 minutes; and to 95° C. (203° F.), 48 minutes. The 40 gallons of milk, skim milk or whey were cooled by pumping over a surface cooler at the rate of 2 gallons per minute. From the other hotwell the 50 gallons of milk were drawn into a vacuum pan in 6 minutes, where 10 minutes elapsed before the temperature of the vapor over the boiling milk was lowered to 40 (104° F.) to 45° C. (113° F.).

To determine the effect of a preheating treatment on the rate of development of milkstone, the preheated material was heated in the clean, high-

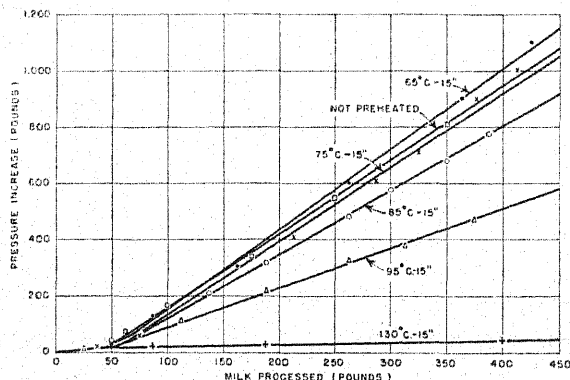


FIG. 1. Effect of preheating treatment of whole milk on the rate of pump pressure increase due to milkstone formation when the preheated milk was heated at 130° C. for 15 seconds by pumping it through heating, holding and cooling coils of 0.18 inch internal diameter. Preheating treatment is shown on each curve. Rate of milkstone formation is expressed as pounds per square inch increase in resistance to flow per pound of milk processed.

temperature equipment in 3 seconds to 130° C. (266° F.), maintained at this temperature for 15 seconds, and cooled in 3 seconds.

In making this determination, water was heated in the equipment first. When the desired temperature conditions had been established a 2-way valve was turned so that the test liquid, instead of water, would flow to the pump. One minute after this liquid began to pass into the pump the first pressure reading was noted. Additional gage readings were recorded from time to time. The last pressure gage reading was made just before the valve was turned to again admit water. The difference between the first and last readings on the pressure gage was considered the total increase in pressure.

The fresh whole milk was standardized by the Babcock test and a hydrometer reading (2) to a fat:solids-not-fat ratio of 1:2.29.

The whey was the low acid or rennet type. It was prepared from fresh skim milk by the addition of rennet and clarified.

RESULTS

In figure 1 are shown increases in pressure gage readings due to milkstone formation on heating raw milk (not preheated) to 130° C. for 15 seconds and milk to the same temperature and for the same holding period that had been preheated in the high-temperature-short-time equipment at 65, 75, 85, 95, and 130° C. for 15 seconds. As the preheating temperature was increased less solids were deposited on the tubing. When milk that had been heated at 130° C. for 15 seconds was reheated under the same conditions only a small amount of milkstone formed.

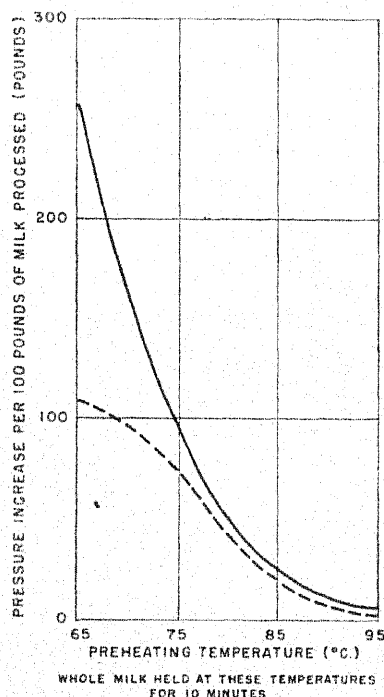


FIG. 2. Effect of preheating treatment of whole milk on the rate of pump pressure increase due to milkstone formation when the preheated milk and condensed milk made from it were heated at 130° C. for 15 seconds by pumping them through a high-temperature tubular heater. Solid line represents whole milk, broken line homogenized condensed milk of 26.0% solids content. Rate of milkstone formation is expressed as pounds per square inch increase in resistance to flow per hundred pounds of whole milk processed.

The effect of different preheating treatments of whole milk on the rate at which milk solids adhered to the tubing is shown in figure 2. In these experiments both fluid whole milk and its concentrate of 26 per cent solids content were used. The concentrated milk was homogenized at 60° C. (140° F.) and 2,500 pounds pressure before the final or test heating at 130° C. for 15 seconds.

As the preheating temperature was increased the rate at which the tubing became coated with solids decreased until, with a preheating temperature of 95° C. and a holding period of 10 minutes, the rate was slow.

In terms of whole milk equivalents, solids in the concentrated milks adhered to the tubing at a slower rate than did the solids in the preheated whole milk. However, in terms of pounds of concentrated milk processed

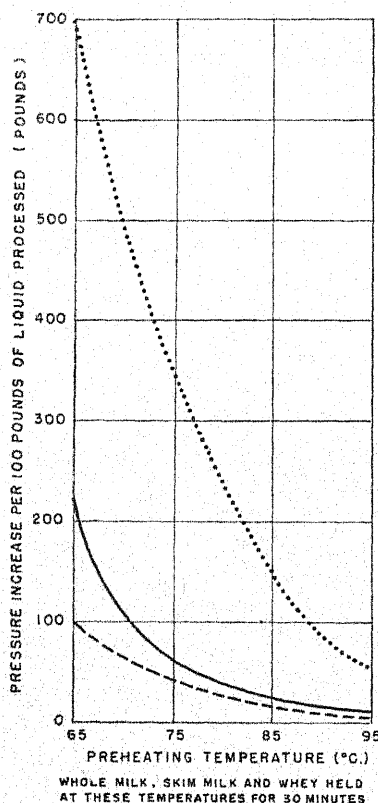


FIG. 3. Effect of preheating treatment of whole milk, skim milk and whey on the rate at which the pump pressure increased due to milkstone formation when the liquids were heated at 130° C. for 15 seconds. Solid line represents whole milk, broken line skim milk, and dotted line whey.

the rate was much faster except when the preheating temperature was below about 70° C. (158° F.) and above 90° C. (194° F.)

In obtaining the data shown graphically in figure 3 the same procedure was followed as in obtaining the data presented in figure 2 for whole milk except that skim milk and whey were used as well as whole milk and the holding period at each preheating temperature was 30 minutes.

DISCUSSION

The formation of milkstone on dairy equipment has received much attention. It makes the transfer of heat more difficult; the deposit is insanitary, is deleterious to the metal, and must be removed. Shere (5) has described this deposit and recommends methods for removing it. He shows that its composition varies but that it is organic matter mixed with small quantities of substances which contain calcium and phosphorus.

An effective procedure for cleaning the tubing of the high-temperature-short-time equipment was to recirculate continuously through it a solution of hot trisodium phosphate and then, after flushing with water, a solution of citric acid. The pH of the former was about 11.5 and of the latter, about 2.0. In this way a large reduction in pressure was obtained in a few minutes and then, when the acid was recirculated continuously, soon there was a return to the pressure which was normal for forcing the liquid through clean tubing. If the acid was used first, only a small reduction in pressure was obtained, and usually only after a relatively long time. Finally when the acid was followed by the alkali, the tubing was cleaned quickly. This indicates that the milkstone was largely organic mixed with small quantities of inorganic matter. The proteins of milk are more soluble at pH 11.5 than at pH 2.0 and calcium phosphates are soluble at pH 2.0 but not at pH 11.5.

It is interesting to compare the relationship between the preheating treatments and the formation of milkstone with the heat denaturation of the soluble milk proteins, albumin and globulin.

Rowland (3, 4) made an investigation of the amounts of lactalbumin and lactoglobulin denatured (rendered insoluble) by heating portions of the same milk for varying periods at each of several temperatures. He found that appreciable quantities of albumin and globulin are denatured at as low a temperature as 63° C. (145° F.). In the summary of his first article he says, "Smooth curves were obtained for the progress of denaturation with time at each temperature, and, over the range of 63-75° C., the relative increase in velocity of denaturation for each rise in temperature of 1° C. was found to be constant, the temperature coefficient of the reaction being 1.5."

In the summary of his second article Rowland states, "The denaturation of albumin and globulin took place rapidly in samples of milk heated at temperatures of 75° C. and above, and was complete in approximately 60, 30, 10-15, and 5-10 minutes at 80, 90, 95, and 100° C., respectively."

Bell (1) studied the effect of heat on the solubility of the calcium and phosphorus compounds in milk. From his results he concluded that "... there is a loss in the soluble calcium and phosphorus contents of the skim milk due to heat and that the amount of the loss depends upon the temperature to which the milk has been heated.

"The results from the methods employed indicate that definitely measurable amounts of these substances are removed from solution in milks heated to 170° F. or above."

In view of the time-temperature conditions required to denature all the albumin and globulin in milk as shown by Rowland, the continued removal of calcium and phosphorus from solution due to heat as reported by Bell, and the results described in this paper, it seems probable that the formation of milkstone in a high-temperature-short-time heater can be practically prevented by suitable preheating of milk, skim milk and whey.

SUMMARY

1. The formation of milkstone in a high-temperature-short-time heater was greatly decreased by preheating milk, skim milk and whey.
2. There appears to be a direct relationship between the heating conditions which render insoluble some of the proteins and salts of milk and the prevention of milkstone formation.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

1. **Food Enough.** JOHN D. BLACK. The Jaques Cattell Press, Lancaster, Pa. 1943. \$2.50.

In addition to including basic facts on the need for food and food nutrients, this book analyzes the world's food supplies and the nutritional status of various peoples, points out the problems of war time feeding, considers production of food on the basis of most effective utilization of natural resources, suggests policies that would best cope with the present food and feed crisis, and recommends a course of marketing procedure designed to overcome much of the difficulties arising between the points of production and ultimate consumption.

Included also are excellent discussions of the need for and mechanics of rationing, shifts in dietary patterns, industrial feeding, and the world-wide significance of the United Nations Conference on Food and Agriculture.

The author is Henry Lee Professor of Economics at Harvard University, a member of the Economic Panel of the Interim Commission of the United Nations Food Conference, and a member of the Food and Nutrition Board of the National Research Council.

The importance of food and the relationships between food supplies and the course of world events now and in the future make this volume of great timeliness. Written by an economist with much practical experience and with many associations that place him in a position to know the facts, and written in a style that is easily read by anyone, *Food Enough* should be on the "must" list for all. Agriculture in general and dairying in particular are given encouraging places in future world economy. W.E.Krauss.

BACTERIOLOGY

2. **Bacteriophages for *Streptococcus cremoris* Phage Development at Various Temperatures.** G. J. E. HUNTER, Dairy Res. Inst., Palmerston North, New Zealand. Jour. Dairy Res., 13, No. 2: 136-145. 1943.

The effect of temperature on the growth in milk of several strains of *Streptococcus cremoris* and their appropriate phages was investigated. Acid formation and rate of phage multiplication were followed in 100-ml. quantities of autoclaved skim milk seeded with 1% of an 18-20-hour clotted-milk culture and varying amounts of phage added. At intervals 2 ml. were withdrawn for acidity titration and 1 ml. was used for dilution in the plate test for phage multiplication.

In general, the streptococci grew more rapidly at 30° C. (86° F.) than at 22° C. (71.6° F.) but were inhibited to a greater or less degree at 37° C. (98.6° F.). The phage races for the most part developed somewhat more readily at 30° C. (86.0° F.) than at 22° C. (71.6° F.). Some developed just as readily at 37° C. (98.6° F.) as at 30° C. (86° F.) but others were completely inhibited at 37° C. (98.6° F.).

These results suggest that the phage races as they exist are separate and distinct entities whose growth conditions are similar to, but not necessarily identical with, those of the bacteria upon which they develop. S.T.C.

3. Distribution of *Flavobacterium maloloris*. R. M. REYNOLDS AND H. R. THORNTON, Univ. of Alberta, Canada. Sci. Agr., 24, No. 1: 21. 1943.

Workers in this laboratory had previously found *F. maloloris* to be an infrequent cause for surface taint in butter. In this study 589 isolations of yellow bacteria from 140 samples of Alberta water were examined and only two proved to be *F. maloloris*. O.R.I.

4. The Occurrence of Slow-Reducing Coliform Organisms in Milk. C. S. MORRIS, Seale Hayne Agr. Col., Newton Abbott, Devon, England. Jour. Dairy Res., 13, No. 2: 115-118. 1943.

Milk samples were found frequently to contain coliform organisms which give very slow reduction of methylene blue at 37° C. (98.6° F.). Evidence was secured which indicates that this slow reduction is due to two factors present in raw milk, both of which are destroyed by heating milk to 70° C. (158° F.) for one hour. These factors are (a) a specific bactericidal substance and (b) a growth-inhibiting factor.

All of the cultures examined appeared from differential tests to be intermediate types of coliform organisms. All were citrate negative and failed to produce acid or gas when grown in MacConkey's broth at 44° C. (111.2° F.). S.T.C.

BUTTER

5. Churn Washing Procedure. S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 5, No. 2: 120-122. 1943.

Dry rot of staves of the churn barrel is caused by fungi which could be destroyed by heat if it were possible to sufficiently heat all parts, around bolts, crevices, and corners. Cypress is less susceptible to dry rot than Douglas fir. The treatment of churns with hot water apparently neither weakens the wood nor favors dry rot. The most satisfactory procedure of washing the churn appears to be, first, a rinse with water at 120-140° F. to remove the bulk of the fat; second, running the churn for 15 minutes half full of water at 180° F. containing a mild alkaline washing powder; and,

third, running the churn for 15 minutes half full of water at 200° F., draining thoroughly, and allowing to dry with the doors open. Reduction in the temperature of the water does not accomplish the purpose or prolong the life of the churn. P.S.L.

6. **Water Supply Difficulties.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 5, No. 2: 46-48. 1943.

Where raw water used as wash water for butter causes off flavors due to bacterial contamination and chlorinated water does the same due to its chemical content, the ideal procedure is to dechlorinate the water before use. The ultra violet ray water sterilizer is of little value for this purpose. The use of small amounts of chlorine causes more off flavor due to chlorophenol and similar compounds than does large amounts of chlorine. Many creameries successfully use wash water containing 25 p.p.m. of chlorine. The writer describes a method for construction of a dechlorinating filter.

P.S.L.

7. **Court Cases Involving Butter.** LEO T. PARKER, Attorney at Law, Cincinnati, O. Amer. Butter Rev., 5, No. 1: 8, 10, 12. 1943.

The author lists several legal interpretations, decisions, and rulings concerning butter; the validity of state laws; public policy, monopoly, trade mark, and ceiling price laws; and validity of contracts. Each is illustrated with recent court decisions.

P.S.L.

8. **Moisture Loss in Prints—Combatting Leaky Bodied Butter.** S. T. COULTER, Univ. of Minn., St. Paul, Minn. Amer. Butter Rev., 5, No. 3: 86. 1943.

Due to reduced water retaining powers winter butter moisture standards should be reduced if leakiness is to be prevented. Such procedure as reduces size of the water droplets helps in reduction of leakiness. These include working the butter while firm, use of 40° F. wash water, filling the churn no more than 40% full, prevention of stickiness of the churn, and adding all water of standardization at the time of adding the salt.

P.S.L.

CHEESE

9. **Bacteria, Environment and Cheese.** E. G. HASTINGS, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., 34, No. 10: 14. Oct., 1943.

Its environment determines the fate of every living thing—although man cannot always recognize how it does so. The cheesemaker seeks to establish in milk, curd and cheese a desired bacterial pattern by controlling tempera-

ture, acidity, moisture and salt. His sensitivity to the milk is most important in the making of uniform cheese. The uniformity expected by many is unattainable but can be best achieved by the use of milk from the same farms daily, to the bacterial pattern of which the maker has adapted himself. A change of milk or maker can be expected to change quality. Pasteurization of milk simplifies the bacterial pattern and though it slows flavor development it may be possible to find ripening agents to remedy this effect. If normal, three or four months old pasteurized-milk cheese is ground and stirred thoroughly, then flavor development approximates that in raw-milk cheese. The few essential, flavor-producing bacteria which survive pasteurization are scattered by this mixing, so that they reach the food they need and quickly produce flavoring substances. Ground cheese can also be blended for uniformity and reformed and packaged if provision is made for the venting of CO_2 formed by the continued action of ripening agents. Occasionally pasteurized-milk cheese cures normally without CO_2 development which suggests new ripening possibilities if it were understood. Too little attention has been given to producing the smooth, waxy body desired by most consumers; too much attention has been given to eye formation in Swiss. "Eyes in a woman or a Swiss cheese are something to consider but not to control one's judgment as to desirability." W.V.P.

10. The Effect of Over-Ripening upon the Activity of Cheddar Cheese Starters. C. K. JOHNS AND H. L. BERARD, Div. of Bact. and Dairy Res., Sci. Serv., Dept. Agr., Ottawa, Canada. Jour. Dairy Res., 13, No. 2: 127-135. 1943.

In a study involving three starters, over-ripening brought about by increasing the amount of inoculum, by lengthening the period of incubation, or by increasing the temperature of incubation, failed to slow down the rate of bacterial growth, or acid development, or to lower the final acidity reached. Starters repeatedly over-ripened, over a period of 30 days were considered superior in flavor to those normally ripened.

In practical cheesemaking an over-ripened portion of a starter worked slightly faster in the vat and produced cheese with a higher flavor score than the control. S.T.C.

11. The Influence of Abnormal ("Non-Acid") Milk on Cheese Starter Cultures. G. J. E. HUNTER AND H. R. WHITEHEAD, Dairy Res. Inst., Palmerston North, New Zealand. Jour. Dairy Res., 13, No. 2: 123-126. 1943.

"Non-acid" milk is described as a colloquial term coined by cheesemakers in New Zealand to designate milk which hinders the development of acid by a normally active starter culture in the cheese vat. Stock cultures of "non-acid" organisms were grown at 30° C. (86° F.) in sterilized skim milk for

24 hours. The acidity rise was 0.03–0.06 per cent expressed as lactic acid. This “non-acid” skim milk was mixed in varying proportions with normal fresh milk and the resulting mixture used for starter propagation. Delayed coagulation simulating a starter failure caused by bacteriophage was demonstrated. Some strains of starter cultures were considerably less inhibited than others.

S.T.C.

12. **Further Studies on Bacteriophage in Relation to Cheddar Cheese-making.** C. K. JOHNS, Div. of Bact. and Dairy Res., Sci. Serv., Dept. Agr., Ottawa, Canada. *Jour. Dairy Res.*, 13, No. 2: 119–122. 1943.

Trouble with slow working vats in two factories which had continued over a period of six weeks was shown to be due to lysis of the dominant strains of the mixed culture of starter organisms by phage. Normal acid development was secured by substitution of a starter of entirely different bacterial strains.

A second outbreak of phage infection with complete cessation of acid production in experimental vats is described. The starter itself appeared to be entirely free from phage and the equipment had been drastically sterilized following a previous outbreak. The original source of the phage was not definitely demonstrated although occasional positive indications were obtained from the milk supply itself.

S.T.C.

13. **The Production of Rennet from Living Calves.** N. J. BERRIDGE, J. G. DAVIS, P. M. KON, S. K. KON, AND F. R. SPRATLING, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. *Jour. Dairy Res.*, 13, No. 2: 145–161. 1943.

Abomasal juice containing rennin was obtained from living calves by means of an abomasal fistula. The operation for fistula was performed on two calves at 14 days of age. The animals received, during the three months for which the experiment lasted, an exclusive diet of whole milk supplemented by minerals and vitamin D.

Abomasal juice was obtained by allowing the calves to drink dilute whey and removing it through the fistula in about half an hour. The mean yield of rennet for each “perfusion” from the first calf was 3120 units with a standard deviation of ± 1330 and 5680 units with a standard deviation of ± 2560 from the second calf. One unit of rennet is defined as the amount sufficient to coagulate in 100 seconds 10 ml. of a substrate consisting of 12 grams of spray-dried skim milk in 100 ml. of N/50 calcium chloride solution.

A concentrated rennet was prepared from the abomasal juice. Cheese made from the fistula rennet was indistinguishable from the control cheese.

This method of rennet production was regarded as too expensive in time, labor, and cost of food for use in commercial rennet production in England.

S.T.C.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

14. Relation of Lecithin to the Keeping Quality of Dry Whole Milk.

C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State College.
Natl. Butter and Cheese Jour., 34, No. 10: 18. Oct., 1943.

Fresh milk was separated into cream and skim milk. The cream was churned and the butter melted and washed to obtain pure butter oil. A portion of the skim was centrifuged at 25,000 RPM to reduce the lecithin in the skim. Samples of the fresh milk, pure butter oil and skim, and pure butter oil and centrifuged skim were pasteurized, homogenized, concentrated 2 to 1, and dried by the atmospheric roll process. The dry milk was placed in brown bottles. Half of each lot was charged with nitrogen, and all containers were carefully sealed and stored at 85° F. Some of each was reconstituted and examined at monthly intervals. The samples with least lecithin kept best although all samples deteriorated. Over half of the milk lecithin remained in the skim milk and though centrifuging reduced the amount, it may also have removed some other pro-oxidant. The experiments indicate the possibility of producing dry milk with excellent keeping qualities.

W.V.P.

15. The Gas-Packing and Storage of Milk Powder. C. H. LEA AND T.

MORAN, Low Temp. Res. Sta., Cambridge, England, and J. A. B. SMITH, Hannah Dairy Res. Inst., Ayr. Jour. Dairy Res., 13, No. 2: 162-215. 1943.

The authors present the following summary of their extensive work:

“(a) Full-cream powders stored in the presence of up to 0.01 ml. of oxygen per g. of powder kept very well at both normal and high temperatures. This figure corresponds to 1% oxygen (after completion of desorption) in the free-space gas of a can of spray-dried powder packed to a bulk density of 0.55 g./ml., or to 0.5% of oxygen in a can of roller powder packed to a bulk density of 0.35 g./ml. Tallowiness was never definitely detected under such conditions, and there seems to be little or no advantage to be gained, at least so far as palatability is concerned, by improving on this figure. An atmosphere containing not more than 0.01 ml. of oxygen per g. of powder can therefore be considered an ideal pack for milk powder.

“(b) Powders stored in the presence of 0.02 ml. of oxygen per g., *i.e.*, approximately 2% of oxygen in the free-space gas of a can of spray-dried full-cream powder, also kept well, in fact when storage was at 15° C. (59° F.) it was often impossible to distinguish between such powder and others stored in the presence of much smaller amounts of oxygen. At the high temperature at 37° C. (98.6° F.) the powder was usually, but not invariably distinguishable from powders stored in lower concentrations of oxygen, but the difference was slight. Below 0.02 ml. of oxygen per g. of powder may therefore be considered a good commercial pack.

"(c) Powder stored in the presence of 0.03 ml. of oxygen per g., *i.e.*, approximately 3% of oxygen in the free-space gas of a can of spray-dried full-cream powder, kept quite well. The testing panel could usually distinguish between powder stored for long periods at 15° C. (59° F.) in this and in lower concentrations of oxygen, but the difference was still small and probably not important from a practical point of view. In view of the difficulty of gas-packing, spray-dried powders in very low concentrations of oxygen, values up to 0.03 ml. of oxygen per g. of powder can be accepted as satisfactory for a commercial pack. It should be possible to store full-cream powder under those conditions for several years without serious deterioration. A rather lower concentration of oxygen than 0.03 ml./g. might, however, be desirable if storage is to include exposure to high atmospheric temperatures.

"(d) Powder stored in the presence of 0.04 ml. of oxygen per g., *i.e.*, approximately 4% oxygen in the free-space of a can of spray-dried full-cream powder, developed a suspicion of 'off' flavor (mark 1.0) in about 7 months and a definite slight 'tallowy' odor and flavor ('off' flavor mark 1.5-2.0) in about 12 months at 15° C. (59° F.), but was little, if any, worse after 2 or 3 years' storage. Though always considered usable and sometimes quite good, such powder was definitely inferior to samples stored in lower concentrations of oxygen, and so high a figure as 0.04 ml./g. should not be accepted as satisfactory. At 37° C. (98.6° F.) results were more unfavorable than at 15° C. (59° F.) and a sample of spray full-cream powder stored at this temperature was considered to have become unusable for part of the storage period, between 4 and 12 months, when 'off' flavor marks between 2.0 and 2.8 were recorded. The powder subsequently improved slightly to mark 2.0 at 12 and 16 months."

S.T.C.

DISEASE

16. **Variations in the Occurrence of Bloat in the Steer Progeny of Beef Bulls.** B. KNAPP, JR., A. L. BAKER AND R. W. PHILLIPS, U. S. D. A., Beltsville, Md. Jour. Anim. Sci., 2, No. 3: 221-225. Aug., 1943.

The progeny of 13 registered Hereford bulls were used over a two-year period in this study. A highly significant difference existed between progeny groups in both years on the frequency of bloat. The results indicate that there are inherent differences between progeny of different bulls in ability to handle large quantities of feed without digestive disturbances and indicate the possibility of improvement in this characteristic by selection on the basis of progeny tests.

C.F.H.

FEEDS AND FEEDING

17. **Determinations of Metabolizable Energy of Feeding Stuffs for Cattle.** E. B. FORBES AND E. J. THACKER, Dept. Anim. Nutr.,

Pennsylvania State Col., State College, Pa. Jour. Anim. Sci., 2, No. 3: 226-230. Aug., 1943.

In working out a method for calculating metabolizable energy from the digestible nutrients, a comparison was made between experimentally determined metabolizable energy of a group of feeding stuffs and computed values according to the formula of (1) Bratzler, (2) Kriss and (3) Axelsson. Metabolizable energy values calculated according to the method of Axelsson were the most satisfactory for feeds other than silage. When the factor 3.3 was used in computing metabolizable energy of the digestible ether extract of silage along with Axelsson's factors for digestible protein and carbohydrates of roughages, total metabolizable energy values of silages were in approximate agreement with values based entirely on experimental observations.

C.F.H.

18. Die Eignung von Kartoffeleiweisspülpe als Milchviehkraftfutter.

J. SCHMIDT AND J. KLIESCH, Univ. of Berlin. Züchtungskunde, 18, No. 2: 43-49. Feb., 1943.

Two groups of six cows each were fed 3 kg. per day of this protein pulp to replace an oil cake mixture. The results were unfavorable. This was repeated in another three-week experimental period. When the experiment was again repeated but with only 2 kg. per day, practically no difference between the two groups was obtained. It is tentatively concluded that this feed may be fed with good results if the rate is not in excess of 2 kg. per day.

J.L.L.

19. Untersuchungen über die Verwertung von Harnstoffstickstoff durch wachsende Kälber. J. SCHMIDT AND J. KLIESCH, Univ. of Berlin. Züchtungskunde, 18, No. 1: 1-11. Jan., 1943.

Whether the nitrogen in urea could be used by growing calves to replace part of their protein needs was studied on two pairs of identical twin calves and in another experiment with three lots of five calves each. The first pair of twins was fed for 728 days. The data are presented for them in five consecutive periods. The second pair was fed for 436 days and the results are presented separately for four periods of this time. The three groups were fed over a period of 252 days. The basic ration was clover hay and dried beet pulp. The control groups received in addition a concentrate mixture thought adequate to cover their protein needs. The amounts of digestible crude protein ingested per day were the same, but the digestible true protein was 390 g. per day for the control group and only 190 g. per day for the amide group. The third group was given only as much digestible true protein as the amide group and barely over half as much digestible crude protein. The growth curves of the control group were far above those

of the other two. The growth curve of the group receiving the amide nitrogen was only a little above that of the group which received the same amount of true protein but no extra amide nitrogen. The authors conclude that both sets of experiments show that growing calves are not able to use the nitrogen of urea to any practical extent for growth. This agrees with their earlier and more extensive experiments on sheep. J.L.L.

FOOD VALUE OF DAIRY PRODUCTS

20. **Reviews of the Progress of Dairy Science. Section D. The Nutritional Value of Milk and Milk Products.** S. K. KON, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. *Jour. Dairy Res.*, 13, No. 2: 216-241. 1943.

This is an excellent review of the literature on the nutritional value of milk and milk products covering the period from the beginning of 1940 to the end of 1942. 430 references. S.T.C.

ICE CREAM

21. **Dried Whole Egg Powder. V. Definition and Properties of Low Grade Powders.** M. W. THISTLE, MARGARET REID AND N. E. GIBBONS, Natl. Res. Council of Canada, Ottawa. *Canad. Jour. Res.*, D, 21, No. 8: 267. 1943.

The point at which 50% of a panel of tasters regarded dried egg powder as unsuitable for human consumption coincided with a rating of 2.7 on a scale ranging from 10 for excellent, fresh egg, to 0 for repulsive material. The protein fraction of these low grade samples had deteriorated badly, as shown by fluorescence measurements. The fat fraction showed no evidence of peroxide oxygen formation however. O.R.I.

22. **Better Ices and Sherbets.** C. D. DAHLE, Pennsylvania State Col., State College, Pa. *Ice Cream Trade Jour.*, 39, No. 10: 30. Oct., 1943.

The author outlines the requirements for, and characteristics of, high quality sherbets and ices, pointing out the difficulties encountered in manufacture under present conditions. Particularly stressed are the necessity for controlling the overrun under 50%, preferably at about 35%; the use of corn sugars high in dextrin and such cereal products as oat and wheat flour for improving body and texture; the proper amount of sweetening from the standpoint of body and texture as well as flavor; and the necessity of considering the sugar content of fruit products used as flavors. Defects of ices and sherbets are also briefly considered. F.J.D.

23. **Wartime Trends in the Retail Ice Cream Store.** CHARLES PAINO, Natl. Assoc. Retail Ice Cream Mfgs. *Ice Cream Trade Jour.*, 39, No. 10: 26. Oct., 1943.

Results of a survey conducted among 80 companies representing 300 retail ice cream stores revealed that sugar and labor are the most important problems facing operators of stores. Only 2% of the stores have discontinued the sale of cones and in these cases only intermittently. Eighty per cent have discontinued the sale of bulk carry-out ice cream and the other 20% have done so at times, but only 2% have discontinued the sale of packages. Sales of sherbet have ranged from 10% to 50% of the total gallonage with the big majority reporting about 25%. Over half the stores report that the consumer does not like to be forced to buy sherbet with ice cream. All of the stores are confident that they will be able to continue in business during the war. F.J.D.

24. **Possible Conversion of Ice Cream Plants to Frozen Foods.** F. L. THOMSEN AND RICHARD GABEL, Bur. Agr. Economics, U. S. D. A., Washington, D. C. *Ice Cream Trade Jour.*, 39, No. 10: 38. Oct., 1943.

This article is the first report of a survey being conducted with the object of determining the practicability of utilizing the resources of the ice cream industry for the freezing and distribution of frozen fruits, vegetables, meats and fish should future conditions in the canning industry demand it. The possibilities of a shortage of tin, should the war with Japan be long drawn out, are pointed out as are also the lack of refrigerator cars and the fact that greater utilization of fresh products is difficult. Some arguments for conversion of a small proportion of the ice cream industries' facilities at an early date rather than a substantial conversion at a later date are advanced. The general theme of the article seems to be that it would be better to play safe by conserving the available tin supply in some such manner rather than to hope for the best and be left high and dry with no tin for even the essential uses. F.J.D.

MILK

25. **Dipper Strainer—Flaky Milk.** H. J. BRUECKNER, Cornell Univ., Ithaca, N. Y. *Amer. Milk Rev.*, 5, No. 1: 10. 1943.

The dipper-strainer, with fine mesh screen bottom, is being used rather successfully at milk stations for detection of mastitis milk. Flakes of fat may be distinguished from curd flakes, and are caused by improper handling of milk at the farm, chiefly by air cooling or slow cooling, mixing warm with cooled milk, and cooling milk in tanks not sufficiently filled with water to

reach the surface of the milk in the can. This method for examination of milk gives promise of much wider adoption.

P.S.L.

26. **Prospects for Milk Supplies in 1944.** T. G. STIRTS, Food Distrib. Admin., Washington, D. C. Ice Cream Trade Jour., 39, No. 10: 22. Oct., 1943.

Indications are that milk production in 1943 will be slightly less than in 1942 and estimations for 1944 are less than for 1943. Serious shortages of butter, cheese and fluid milk have developed in many areas. These are attributed to increase in the consumption of fluid milk and the greater utilization of dairy products by the armed forces and for Lease-Lend purposes. Recent Order No. 79 aims to prevent further increase in the consumption of fluid milk in order that supplies will be available for butter, cheese, and concentrated products. Notwithstanding Order No. 8 which reduced the milk solids going into ice cream by 35%, the total gallonage of frozen dairy foods for 1943 will be no less than in 1942, although it is estimated that the equivalent of 100,000,000 pounds of butter and 60,000,000 pounds of dry skimmilk will have been saved.

The outlook for 1944 is that milk and dairy products will fall short of supplying demands. Subsidies of 25 to 50 cents per cwt. of milk to farmers and 3 to 6 cents per pound of fat for cream have been authorized in an effort to stimulate production.

No immediate change in the quota of milk solids for ice cream purposes is contemplated, but should the demand for milk solids for other, more urgently needed, dairy products become increasingly unsatisfied a review of existing food orders affecting dairy products would become necessary.

F.J.D.

ABSTRACTS OF LITERATURE

BOOK REVIEW

27. **Drying and Dehydration of Foods.** HARRY W. VON LOESECKE, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. Published by Reinhold Publishing Corporation, 330 West Forty-second Street, New York. Consists of 10 chapters, glossary of terms, patent list, index. 302 pages. \$4.25.

The tremendous wartime use of dehydration as a means of enabling efficient preservation and movement of foods in the light of packaging and transportation problems has led to many developments in the art. The dairy industry has experienced a phenomenal increase in dehydration in its own field. A knowledge of the processes and developments in other food product fields is important to workers in the dairy industry because of a potential utilization of milk in dehydrated processed foods. The book, *Dehydration of Foods*, is written in an excellent review manner, devoid of quotations and citations. The references necessary to the subject are listed separately at the end of each chapter. Types of dehydrators includes illustrations of the principal types of units employed in the dehydration industry. A short resume is included in this chapter on the air, heat, moisture relationships of a tunnel dryer. Other chapters are: Sun drying and dehydration of fruits; Dehydration of Vegetables; Dehydration of Eggs; Milk and Butter; Dehydration of Meat, Fish, and Beef Blood; Plant Sanitation; Costs of Dehydration; Nutritive Value of Dried and Dehydrated Foods; Packaging and Storage; Methods of Analysis, and Reconstitution of Dehydrated Foods. The section on dehydration of milk contains no information not available in recognized dairy texts. No discussion is included of specific techniques and problems of roller drying of skim and whole milk, nor of the quality standards for dried milk. The material in this chapter is brief in the light of the importance of the industry. The discussion in the text is well organized, and easily readable, and enables a ready grasp of principal methods in food dehydration. It will be an excellent book for those in the dairy industry seeking a good review of other branches of the food dehydration industry, and their methods.

K.G.W.

BREEDING

28. **An Analysis of Milking Shorthorn Records.** W. L. GAINES. Ill. Agr. Expt. Sta. Bul. 498. Aug., 1943.

Because milk records for Milking Shorthorn cows had never been analyzed in the same way as those for other breeds, data from Volumes 9 to 23 (1924-1938) of the Milking Shorthorn Year Book were collected and studied. The records were studied as a whole and in groups according to

number of milkings daily, length of calving interval, and length of record. Only records which included age of cow at calving, length of record, milk-fat yield, and fat percentage in addition to milk yield were studied. Milk-energy yield was computed by the usual formula. For the 6,311 records the average yield was 8,337 pounds of milk, 330 pounds of fat, and 8,285 pounds of FCM, and the average fat percentage was 3.97. The subgroups differed greatly in average milk yield, milk-fat yield, and FCM yield, but differed very little in average fat percentage. For the records as a whole, the correlation between fat percentage and milk yield was -0.217 ; between fat percentage and fat yield, $+0.106$; and between fat percentage and FCM yield, -0.026 (not significant). Similar correlations were found in each of the subgroups. When the change in yield between 3.0% fat and 5.5% fat was expressed by a straight line, milk yield showed a decrease of about 30%, fat yield increase about 30%, and FCM showed very little change. These records afforded an opportunity to check the age-correction factors previously used for Milking Shorthorns, which were based on records of the breed up to June 1, 1920. The records reported here show a distinct shift toward earlier maturity, amounting to 6 months; and the age-correction factors need to be adjusted accordingly. There is no way of knowing whether this earlier maturity represents a change in the dairy qualities of the breed or a change in management of the cows. Actually age correction is probably simply an indirect allowance for live weight increases with age. A system of milk-yield correction based on live weight would be biologically more sound than an age-correction system, at least for cows less than 13 years old. The season in which a cow calved had an appreciable effect on FCM yield. In general August calvers had the lowest yield and November calvers had the highest. Certain of the records were studied to discover the difference between FCM yields of cows milked three times a day and of cows milked twice a day. The records of a typical group showed that cows milked three times a day exceeded in yield those milked twice a day by 39%. The standard for dairy cows of the Bureau of Dairy Industry is that cows milked three times a day should outyield those milked twice by 24%, so it appears that Milking Shorthorns respond well to the more favorable conditions associated with three milkings daily. A new table of age-correction factors for the Milking Shorthorns is given together with notes explaining its adaptation for use with other dairy breeds.

11 figures, 8 tables.

J.G.A.

BUTTER

29. The 50-45-40 Method of Making Butter. G. H. WILSTER, Oreg. State Col., Corvallis. Natl. Butter and Cheese Jour., 34, No. 11: 16. Nov., 1943.

See Abstract No. 534, page A229, Vol. 26, No. 12, Dec., 1943.

W.V.P.

CHEESE

30. **What About the Tentative New U. S. Cheese Grades?** WALTER V. PRICE, Univ. of Wis., Madison. *Natl. Butter and Cheese Jour.*, 34, No. 11: 8. Nov., 1943.

The tentative U. S. grades on cheddar cheese are unnecessarily complicated in defining grades for the primary market and unjustified in extending similar definitions and grade names to the consumer trade in cured cheese. These tentative grades should be carefully studied by graders, trade associations, consumer groups and commercial organizations and public hearings should be held by the Food Distribution Administration to review and, if necessary, revise these grades. W.V.P.

31. **Milk Filtering in the Cheese Factor.** RAYMOND MIERSCH AND WALTER V. PRICE, Univ. of Wis., Madison. *Natl. Butter and Cheese Jour.*, 34, No. 12: 26. Dec., 1943.

Filtration of milk at the cheese factory does not justify the acceptance of insanitary milk. The capacity of a given filter varies with the condition of the milk, such as its cleanliness, viscosity, acidity, fat and curd content. Preheating of the milk is sometimes used to increase filter capacity. Efficiency of a given filter is affected by the type of pad used, the re-use of washed pads and the volume and the sanitary condition of the milk. Clarification by centrifugal force is being used effectively in the Swiss cheese industry. Costs of filters vary from \$150 to \$600 for commonly used types and up to \$1300 for larger models; filter pads cost from 35 cents to about \$1.50 per day. Manufacturers who try to wash and re-use a filter pad take an unjustified risk. W.V.P.

CHEMISTRY

32. **Pectin as an Emulsifying Agent. Comparative Efficiencies of Pectin, Tragacanth, Karaya, and Acacia.** HARRY LOTZKAR AND W. DAYTON MACLAY. Western Regional Res. Lab., U. S. Dept. Agr., Albany, Calif. *Jour. Indus. and Engin. Chem., Indus. Ed.*, 35, No. 12: 1294. Dec., 1943.

The emulsifying ability of pectin is compared to that of gums tragacanth, karaya and acacia. The importation of these gums has been curtailed by shipping difficulties. The study was made of aqueous emulsions of olive, cottonseed and mineral oils under various conditions of acidity, ratio of oil to water and concentration of agent. Changes in the emulsions were followed by measuring the pH, viscosity, and specific interfacial area of the dispersed oil. In general the tragacanth stabilized emulsions were coarse and viscous, the acacia emulsions were fine and fluid, the karaya emulsions were gelatinous and the pectin emulsions were fine and viscous. The varia-

tion of emulsifying efficiency with acidity and from oil to oil limited generalizations that could be made on the comparative emulsifying efficiencies of pectin and the gums.

B.H.W.

33. **Refractive Index Nomograph for Liquid Fatty Acids.** D. S. DAVIS, Wyandotte Chemicals Corp., Wyandotte, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., 35, No. 12: 1302. Dec., 1943.

Data in the literature covering the variation in refractive index with temperature, in the liquid state, for the normal saturated fatty acids from caproic to stearic, enabled the author to construct the accompanying chart for determining the refractive indices of these acids at any temperature in the range of applicability.

B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

34. **Dry Whole Milk—The Relation of Lecithin to Its Keeping Qualities.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State Col., State College, Pa. Ice Cream Trade Jour., 39, No. 11: 22. Nov., 1943.

The stability of dry whole milk toward the development of oxidized flavor during storage was found to be improved by the partial removal of lecithin normally found in the fat globule adsorption layer. This removal was accomplished by separating the milk, churning the cream and rendering the butter. The resulting butter oil was remixed with the skimmilk before homogenizing, concentrating and drying on a small open roll drier. In one trial the fluid skimmilk was supercentrifuged before mixing with the butter oil. This apparently removed some of the lecithin found in the skimmilk and the dry product exhibited further improvement in keeping quality. Lecithin contents of the dry milks were 0.753, 0.480, and 0.366% (on the basis of the fatty extracts) respectively, for the control product, the butter oil and normal skimmilk product and the butter oil and supercentrifuged skimmilk product.

Improvement in keeping quality of dry whole milk resulting from a partial removal of lecithin was noted in both normal and nitrogen packed containers.

F.J.D.

DISEASE

35. **Septic Sore Throat Epidemic at School.** FRED W. CAUDILL, M.D., M.P.H. Dir., Div. of Communicable Diseases, AND MELVILLE A. MEYER, State Milk Sanit., Bur. of Foods, Drugs and Hotels, State Dept. of Health of Kentucky. Jour. Milk Technol., 6, No. 4: 221. July-Aug., 1943.

A study is reported of two outbreaks of septic sore throat, which occurred among the students, teachers and other employees of the school.

Hemolytic streptococci were cultured from specimens of milk taken from the udders of 3 of the 10 cows in the herd furnishing milk for the school. All of the 10 cows showed the presence of *Staphylococcus aureus* or *albus* in the specimens of milk. The *Staph. aureus* were hemolytic in every instance.

It was thought that one of the boys, who helped with the milking and who reported to the infirmary with a sore throat, which appeared to be an extremely septic throat, was the source of the infection. A period of 7 days had elapsed between his reporting to the infirmary and the occurrence of the first large number of onsets. Improper cleansing and sterilization of milk utensils, milker's hands, cows' udders, and inadequate cooling were thought to be factors contributing to the outbreak.

L.H.B.

36. **Newer Methods for Control of Mastitis.** G. R. SPENCER, Univ. of Wis., Madison. Natl. Butter and Cheese Jour., 34, No. 11: 48. Nov., 1943.

Find infected cows by regular use of strip cups and the Hotis test; segregate them, in separate barns if possible; and use separate teat cups on infected cows. Keep barns clean. Feed less grain to reduce production of infected cows. Milk cows regularly and thoroughly. Medicinal treatments for mastitis as yet are not wholly effective.

W.V.P.

37. **Some Results with Mastitis at Michigan State College.** R. C. HOWOOD, C. F. CLARK, AND C. S. BRYAN. Reprint from Mich. Agr. Expt. Sta. Quar. Bul., 26, No. 1: 43-50. Aug., 1943.

At the start of this program in 1932 there were 56 cows in milk in the college herd. Of these 53.5% had infectious mastitis. In November, 1941, the herd passed its first completely negative test. During the following 13 months to January 1, 1943, there have been nine months without any reacting cows. Acute systemic infection has increased and resulted in an increased loss from death or functional use of the udder. The results in lowering infection in the herd were obtained by culling the infected cows, preventing the spread of the organism, and by recovery by treatment of 16 mastitis-infected animals by lactovaccine and tyrothricin. Thirty-six non-infected cows produced 45% of the milk and 44% of the butterfat from the fore quarters. A reduction of 32% in milk and 32% in butterfat was found in a similar study of 9 cows with opposite-infected and non-infected rear quarters. A comparison of microscopic method, chloride determination, physical examination of udder, leucocyte determination, thymol test, physical examination of milk and culture of milk on blood agar plates, of testing for infectious mastitis on individual quarters, from November, 1932, to February, 1937, on the college herd led to the adoption of the monthly microscopic system of testing. There was no significant variation found in the milk nor udders of three mastitis-infected cows receiving 50

one-hour daily treatments with the short-wave diathermy as compared with three untreated mastitis-infected cows. Lactovaccine was found to correct 26% of the cows positive to streptococci infection. Fifty per cent of the animals responding did so from four treatments, others required up to 12. Treatments beyond that number gave no correction. The study did not indicate that an immunity to infection was developed in non-infected cows. Nine cows treated with tyrothricin became negative to infectious mastitis. A temporary effect which resulted in lower milk production and the production of abnormal milk was observed. Four animals that were treated within three weeks following infection all maintained or showed no increase in production in the lactation following treatment. Only one animal with long-standing infection responded in this manner. Three of the remaining four animals with long-standing infection (10 months to nine years) later developed acute systemic mastitis and died or lost the functional use of the udders. Three other cows in the herd not previously infected were lost during the same period.

J.G.A.

FEEDS AND FEEDING

38. Relationship between Fat Content of Dairy Grain Mixtures and Milk and Butterfat Production. C. F. MONROE AND W. E. KRAUSS. Ohio Agr. Expt. Sta. Bul. 644. 40 pages, illus. Aug., 1943.

Five feeding trials involving a total of 128 cows were conducted in order to determine the effect of different levels of fat in the grain mixtures on milk and butterfat production. The grain mixtures used contained only natural and by-product feeds commonly used in practice. The various fat levels were obtained, with some slight exceptions, by supplementing basal mixtures with either 41% (expeller) soybean oil meal, both with and without ground soybeans, or 44% (browned extracted) soybean oil meal. The continuous type of feeding trial was employed in all five of the trials summarized. With the exception of six Jerseys used in one trial in the second series, the cows were all Holsteins of fairly high milk-producing ability. In the first series of two trials involving 70 cows fed on three levels of fat, the average production of 4% (F.C.M.) milk per 30 days was: pounds, high-fat, 932.1; medium-fat, 921.1; and low-fat, 923.9. In the second series of three trials involving 58 cows fed the two levels of fat, the average production of 4% (F.C.M.) milk per 30 days was: pounds, high-fat, 1,077.5; low-fat, 1,054.3. The butterfat tests and liveweight gains were apparently not affected by the fat levels in the feed. There was also no noticeable difference in palatability in the grain mixtures used. There was no noticeable difference in their effect on the general health and condition of the cows or in preventing or causing udder trouble. Under the conditions of these trials, no significant differences were observed in the production of milk,

butterfat, or 4% (F.C.M.) milk or in the general health of milking cows from the feeding of practical grain mixtures ranging in average fat percentage from 4.89 to 2.69.

19 tables, 5 figures, 23 references.

J.G.A.

39. **The Use of Dried Whey and Blood Meal in the Raising of Calves on Limited Amounts of Milk.** I. L. HATHAWAY, G. W. TRIMBERGER, AND H. P. DAVIS. Neb. Agr. Expt. Sta. Res. Bul. 132. 19 pages. Oct., 1943.

Fifty grade Holstein heifer calves were successfully raised from approximately three weeks to six months of age on alfalfa hay, a grain mixture, a vitamin concentrate, and various amounts of skim milk, supplemented with a mixture composed of 3.2 parts of dried whey to one part of blood meal. Six and eight-tenths pounds of this mixture were used to replace 50 pounds of skim milk. The milk was fed at 50-, 100-, 150-, 200-, 250-, and 300-pound levels. The calves in five of the lots made an average daily gain of approximately $1\frac{1}{2}$ pounds per head for 21 weeks. There was no statistically significant difference among the average gains in weight made by five of the six lots of calves. Three hundred pounds of skim milk alone produced cheaper gains than 50 pounds of skim milk supplemented with 34 pounds of the whey-blood-meal mixture. It was concluded that 6.8 pounds of a mixture composed of 3.2 parts of dried whey and one part of blood meal is a satisfactory substitute for 50 pounds of skim milk in the feeding of healthy dairy calves, which are approximately three weeks of age and which weigh not less than 104 pounds. Thirty-four pounds of this whey-blood-meal mixture can be fed, without serious effects, in 30 days even though as much as 1.8 pounds are fed daily for a few days. Healthy, vigorous dairy calves can be satisfactorily raised from three weeks to six months of age on as little as 50 pounds of the skim milk if it is properly supplemented with dried whey, blood meal, alfalfa hay, a grain mixture, and a vitamin concentrate. Labor can be saved in the raising of dairy calves by replacing the pail feeding of milk over a long period with a few weeks of milk feeding followed by a suitable grain mixture, alfalfa hay, and a vitamin concentrate. With the prices of feeds as quoted herein, the feed cost of raising calves on milk only may be less than when the milk is replaced by this whey-blood-meal mixture. However, when the labor cost is considered in connection with the feed cost, the additional expense of using the substitute mixture will not be prohibitive. Dried whey and blood meal can be utilized as a means of diverting milk from calf feeding to human food and to other uses.

J.G.A.

40. **The Nutritive Value of Korean Lespedeza Proteins and the Determination of Biological Values of Proteins for Growing Dairy**

Heifers. E. W. SWANSON AND H. A. HERMAN. Mo. Agr. Expt. Sta. Res. Bul. 372. 68 pages, illus. Aug., 1943.

An investigation was made of the utilization of the crude protein ($N \times 6.25$) of Korean lespedeza hay and seed and various other feeds by growing dairy heifers. Biological values of the various proteins were determined, and net protein values and other measures of the nutritive value of the proteins were calculated. The net utilization of proteins from lespedeza hay, alfalfa hay, dried skimmilk, corn, lespedeza seed, soybean oil meal, and combinations of lespedeza hay with corn, milk, or soybean oil meal was not significantly different for dairy heifers when they were fed at a 10% level. The feeds were ranked according to the biological value of their proteins as follows: lespedeza hay, corn and lespedeza hay, milk and lespedeza hay, alfalfa hay, corn, soybean oil meal, soybean oil meal and lespedeza hay, lespedeza seed, and dried skimmilk. It was concluded that the quality of the absorbed proteins from Korean lespedeza hay or seed was equal to the quality of the absorbed proteins from milk, corn, alfalfa hay, or soybean oil meal for growing dairy heifers. The digestibility of the crude protein of lespedeza hay was shown to be relatively low, and the high lignin content of lespedeza leaves was revealed as a possible explanation of the poor protein digestibility. Highly lignified late-cut lespedeza hay was shown to be of very low nutritive value, the digestibility of all of its nutrients being greatly depressed. Digestion coefficients were determined for all of the nutrients of intermediate-cut lespedeza hay, late-cut lespedeza hay, and ground lespedeza seed.

12 figures, 16 tables, 110 references.

J.G.A.

HERD MANAGEMENT

41. **Faster Milking.** W. E. PETERSEN. Univ. of Minn. Agr. Ext. Serv. Folder 119. Illus. June, 1943.

The technique of faster machine milking is briefly set forth with reasons for the several steps. Rules are as follows: (1) stimulate the cows to let down their milk one minute before putting on the machine; (2) operate the milking machine according to the manufacturer's directions; (3) strip the cows by machine; and (4) do not leave the machine on the cow after milk stops flowing.

J.G.A.

ICE CREAM

42. **The Preparation and Use of Invert Syrup in the Manufacture of Ice Cream.** E. L. FOUTS, L. E. MULL AND T. R. FREEMAN, Univ. of Fla., Gainesville. Fla. Agr. Expt. Sta. Bul. 393. Sept., 1943.

Invert syrup of 70% solids was made using various acids and heating times. It was found that a satisfactory syrup could be made by boiling for

30 minutes 100 grams of tartaric acid, 100 pounds of sugar and 44 pounds of water. Citric acid in the same amount was found to be equally satisfactory. Three pints of lemon juice in the same formula produced equally satisfactory results and can be substituted for the tartaric and citric acids which are difficult to obtain. The invert syrup will keep well at room temperature.

In ice cream the invert syrup was found to be equal in sweetening value to the same amount of cane sugar and it is recommended that 25 to 50% substitutes of cane sugar can be made in ice cream, but where the latter amount is used the serum solids of the mix should be increased 1%.

C.D.D.

MILK

43. Is It Desirable to Simplify and Unify Our Milk Quality Program.

From the Viewpoint of the Health Officer. N. O. GUNDERSON, Commr. of Health, Rockford, Ill. Jour. Milk Technol., 6, No. 4: 225. July-Aug., 1943.

A four-point test system in use in Rockford, Illinois, is described. This system, after an initial inspection of the farm, shifts the point of observation with reference to quality control from the farm to the receiving dock or city plant. The four-point test used for judging the quality of milk is (1) Sediment test, (2) Direct microscopic (or Reduction test), (3) Phosphatase test, and (4) Swab test, together with other simple observations made at the receiving or processing plant. Under this system farm inspections are limited to the small number of non-compliance milk producers.

The cost of supervising this plan is approximately 0.155 cents per gallon of milk.

Preliminary results of this system of inspection at Rockford for the first five months of 1943 are given.

Some advantages given for this system are as follows:

1. A method of unifying milk control procedures.
2. Quickly locates apparent source of poor milk.
3. A means of culling out low-producing mastitis cows.
4. Minimizes time-consuming routine farm inspection.
5. Emphasizes safety, quality, and flavor of milk—not esthetic scoring of milk control.

6. Is apparently applicable to not only fluid milk, but to cream, butter, cheese, evaporated and dried milk quality control procedures.

7. Conserves manpower so essential in the war effort.

A suggestion is made that a National Allied Dairy Products Council be formed for the purpose of formulating specific recommendations that will simplify and unify the many varied milk control procedures applied to fluid milk, cheese, cream, butter, ice cream, evaporated and dried milk. L.H.B.

44. Homogenized Milk and Public Health. G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., 6, No. 4: 214. July-Aug., 1943.

The history and development of homogenized milk are given together with a very good bibliography on the subject.

Some of the facts regarding homogenized milk and particularly those having a public health significance are discussed. The factors considered are:

1. Homogenized milk must be a pasteurized product. Raw homogenized milk will develop a rancid flavor in a very short time.
2. Homogenized milk may be pasteurized at a higher temperature and maintained for a longer holding period than regular pasteurized milk. There is no cream line on homogenized milk, which will be destroyed by high pasteurization temperatures.

Homogenized milk cannot be mixed with raw milk. Rancid flavor will develop. Only clean milk may be used for homogenizing, otherwise, sedimentation is a possibility. Homogenization, when applied to milk, has a tendency to make the processor more "milk conscious" as it requires more care in processing and merchandising than does unhomogenized milk. Homogenized milk is a factor in developing increased milk consumption, due principally to the fact that homogenization protects against oxidized flavor.

L.H.B.

45. Milk House Construction, Equipment and Maintenance. H. A. BENDIXEN, State College of Wash., Pullman, Wash. Jour. Milk Technol., 6, No. 3: 175. May-June, 1943.

Studies were made at State College on milk house construction and equipment suitable for Washington conditions.

Location, size and construction of milk houses are discussed together with types of materials best suited.

L.H.B.

46. Market Homogenized Milk in Philadelphia. BERNARD SPUR, Milk Res. Labs., Children's Hosp. of Philadelphia, Philadelphia, Pa. Jour. Milk Technol., 6, No. 5: 266. Sept.-Oct., 1943.

That the sanitary quality of the homogenized milk produced in Philadelphia is exceptionally good is shown by the study made at the Milk Research Laboratories, Children's Hospital of Philadelphia, during the eight months period from December, 1940, to September, 1941. Samples were taken each month during this period, with the exception of January and March. The samples were purchased at the distributing platforms of the milk plants.

Of 34 plants producing Grade A homogenized milk, 26 of them had average counts under 8,000. This represented 96% of the total Grade A

homogenized milk consumed in Philadelphia. Only three plants had average counts over 25,000.

There were 36 plants producing Grade B homogenized milk, and of these plants, 22 had average bacterial counts under 8,000. This represented 89% of the Grade B homogenized milk consumed. All but two of the plants had average counts under 50,000 and only one was over 100,000. Twelve of the dairies were known to pasteurize before homogenizing, while three were known to homogenize before pasteurizing.

The bacterial counts obtained in these plants seemed to indicate that the question of getting low counts is more dependent on the sanitary conditions in the plant (and probably on the type of bacteria in the raw milk supply) than on the order of the processes. Of the twelve plants pasteurizing before homogenizing, only one had an average bacterial count over 5,000, and that was an average count of 18,200 on the Grade B milk. The average bacterial count on this plant's Grade A milk was 4,018. Of the three plants pasteurizing after homogenization, two of them had very low counts on both Grade A and B milks (less than 1,400). The other plant had average counts of 15,338 on the Grade A and 11,681 on the Grade B.

The average curd tension of the Grade A milks was 11.6 gms. and on the Grade B was 11.2 gms. The curd tension for the Grade A milks ranged from 5.3 gms. to 18.4 gms., while for the Grade B milks it ranged from 4.6 to 15.9 gms.

L.H.B.

47. **A Ropy Milk Outbreak Caused by a Thermoduric Micrococcus.** C. C. PROUTY, Div. of Dairy Husbandry, Wash. Agr. Expt. Sta., Pullman, Wash. Jour. Milk Technol., 6, No. 5: 263. Sept.-Oct., 1943.

An outbreak of ropy milk occurring in commercially pasteurized milk was studied. An organism closely related to *Micrococcus freudenreichii* was isolated and its source was traced to one of the dairies furnishing milk to the pasteurizing plant. When milk from this producer was excluded, no further ropiness was experienced in the pasteurized product.

Some cultures of the organism were found to survive a pasteurization temperature of 143° F. for 35 minutes, while in other instances 20 minutes at that temperature was sufficient to render the organism nonviable.

The organism had the ability to cause rapid development of ropiness with an abnormal flavor and odor, followed by active proteolysis. L.H.B.

48. **The Connecticut Three-Point Laboratory Program as an Aid to Control of Pasteurized Milk.** FRIEND LEE MICKLE AND EARLE K. BORMAN, Dir. and Asst. Dir., Bur. of Labs., Conn. State Dept. of Health, Hartford, Conn. Jour. Milk Technol., 6, No. 4: 231. July, Aug., 1943.

A three-point program for determining the quality of pasteurized milk

was started in 1937 after a state-wide survey. The tentative standards set were:

- "1. Direct microscopic clump count limits:
Grade A pasteurized milk—200,000 per ml.
Grade B pasteurized milk—500,000 per ml.
2. Coliform organisms: Absent in 0.1 ml.
3. Phosphatase test: Less than 0.05 mg. phenol."

The coliform standard was changed to *absent in 1.0 ml.* in January, 1941.

The use of these standards has not worked a hardship on dealers in good quality milk.

The direct microscopic clump count on pasteurized milk has proven satisfactory in easily classifying milk as either good or bad and with greater rapidity than the agar plate count. In 1939 only 4.1% of the Grade A samples had counts in the borderline range between 200,000 and 300,000, while only 6.5% of the Grade B samples had counts in the borderline range between 500,000 and 1,000,000.

During the five-year period 77.4% of the samples (more than 15,000) met the tentative standards of 200,000 for Grade A and 500,000 for Grade B pasteurized milk by the direct microscopic clump count; 75.6% met the tentative standard for coliform organisms; and 92.7% met the tentative standard for the phosphatase test.

The advantages of the three-point program for determining the quality of pasteurized milk were given as follows:

"1. The direct microscopic clump count presents a more nearly true picture of the bacteriological quality of a milk supply than does the plate count, and provides a much more rapid, more complete, and hence more effective laboratory service to the control official.

2. Tests for coliform organisms, furnishing evidence of improper handling after pasteurization, yield supplementary information correlating well with the direct count.

3. The phosphatase test yields the only positive information obtainable on the effectiveness of pasteurization, and hence is an indispensable index of probable safety of a supply."

L.H.B.

49. Efficiency of Milk Marketing in Connecticut. 5. Economics and Biology of Alternate-Day Milk Delivery. R. G. BRESSLER, JR., E. O. ANDERSON, D. A. CLARKE, JR., AND E. N. BILENKER. Conn. Agr. Expt. Sta. Bul. 247. 60 pages. May, 1943.

Alternate-day delivery of milk has proved to be an effective method of conserving resources. Retail milk truck mileage has been reduced an average of 44% in Connecticut markets. The mileage savings and increased deliveries per stop reduced route time by nearly one-third, which in turn made possibly increased loads and some consolidation of routes. The pro-

gram reduced requirements for gasoline and tires by approximately 40%. Man-hours were reduced 30%, but some of this reduction represented shorter hours per route so that the number of men freed for other employment was only 14% in 1942. Under present conditions annual savings in Connecticut total one million gallons of gasoline, forty-six thousand quarts of oil, and two thousand truck tires, and the elimination of nearly 200 routes.

Monetary savings have not been large in spite of these important reductions in men and materials. In 1941, retail delivery costs in city markets average 4.06 cents per quart. In the spring of 1942 alternate-day delivery costs were approximately 3.70 cents per quart, while a year later, they were 3.61 cents. These savings were offset at least in part by wartime increases in the other costs of milk distribution. If alternate-day deliveries are continued in the post-war period, and if weekly earnings of routemen return to the normal pre-war levels, total delivery costs would be approximately 2.74 cents, a savings of 1.32 cents per quart.

Successful application of alternate-day delivery is dependent on the keeping qualities of milk. Experiments indicate that satisfactory results are obtained where home refrigeration is adequate. P.H.T.

50. Efficiency of Milk Marketing in Connecticut. 6. Truck Costs and Labor Requirements on Milk Delivery Routes. D. A. CLARKE, JR., AND R. G. BRESSLER, JR. Conn. Agr. Expt. Sta. Bul. 248. 39 pages. June, 1943.

In general, costs fell into three categories: overhead costs, fixed operating costs per day, and variable operating costs per mile. Combining overhead and fixed operating costs, the daily costs of operating retail trucks average \$1.68 plus \$0.44 per mile. For wholesale trucks daily costs averaged \$2.46 plus \$0.045 per mile. These costs are based on daily delivery conditions and on cost rates representative of the period 1940-41. The costs of operating alternate-day delivery trucks averaged about \$7.51 per day plus \$0.045 per mile during the spring of 1943.

In greatly simplified form the time required to operate daily retail routes may be represented by 1.2 minutes per quart plus four minutes per mile, while for alternate-day delivery the time requirements are 0.8 minutes per quart plus four minutes per mile. On wholesale routes the time per quart is only 0.35 minutes. Under average conditions of route organization it is possible to handle about 35 quarts per hour on daily retail delivery routes, 60 quarts per hour on alternate-day retail routes and 125 quarts per hour on wholesale routes.

With daily delivery, the average retail driver earned about \$44 per week and labor costs averaged about \$0.026 per quart. With alternate-day delivery average weekly earnings were \$56 (commission basis) while labor costs averaged \$0.025 per quart. On salary and commission payments the

weekly earnings averaged \$46 and costs \$0.025 per quart. On wholesale routes the average cost per quart was between \$0.006 and \$0.007.

In 1941 truck costs averaged \$0.011 per quart, route labor costs \$0.026, miscellaneous costs \$0.004, and total delivery costs of \$0.041 per quart. In 1943 with alternate-day delivery, truck costs averaged \$0.007, route labor \$0.025, miscellaneous route costs \$0.004 and total retail delivery costs \$0.036 per quart. In the spring of 1943 wholesale delivery route truck costs average about \$0.003 per quart, labor costs \$0.006, miscellaneous costs \$0.004, and total delivery costs \$0.014 per quart.

P.H.T.

51. **Efficiency of Milk Marketing in Connecticut. 7. Milk Delivery in Rural Connecticut.** ALAN MACLEOD AND C. J. MILLER. Conn. Agr. Expt. Sta. Bul. 249. 37 pages. July, 1943.

Opportunities exist for conserving scarce resources in the distribution of fluid milk in rural areas. A study of 12 areas in Connecticut has indicated the size of the savings that might be realized from complete adoption of alternate-day delivery and from the allocation of exclusive territories with deliveries made daily or on alternate days.

In the summer of 1942 about 36 per cent of the daily delivery mileage was being saved. This compares with a maximum estimated potential savings of 45 per cent if all producers were placed on an alternate-day basis.

Adoption of a system of exclusive territories would yield potential savings of 38 per cent with daily delivery or of 64 per cent with alternate-day delivery. The present system of alternate-day and daily delivery is saving almost 1.3 million miles yearly in rural Connecticut towns. These savings could be increased to 2.2 million miles by the adoption of exclusive territories in combination with alternate-day delivery. This would produce a savings of about 220,000 gallons of gasoline, 11,000 quarts of oil, and 500 delivery truck tires. The savings in manpower would probably be most important of all as the time released from delivery can be put to more productive uses.

P.H.T.

MISCELLANEOUS

52. **Post-War Problems of the Locker Industry.** SLEETER BULL, Univ. of Ill., Urbana. Natl. Butter and Cheese Jour., 34, No. 11: 22. Nov., 1943.

The rapid growth of the locker industry has resulted in some troubles caused by poor plant design, improper insulation, under equipment, lack of information in management and operation, and consumer ignorance. The future promises more problems such as the development of household units for home freezing and storage and the possibility of freezing foods in transit at high altitudes.

W.V.P.

53. **Pyrex Glass Tubing as a Substitute for Metal Milk Pipe in Dairy Plants.** G. J. HUCKER AND ROBERT E. THOMAS, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Jour. Milk Technol., 6, No. 4: 197. July-Aug., 1943.

A study was made under commercial conditions in a plant handling approximately 45,000 pounds of milk daily.

The study revealed that pyrex glass tubing, beaded or flanged, can serve as a substitute for metal milk pipe in dairy plants.

It proved impractical under general operating conditions to dismantle beaded glass tubing daily for cleansing, the same as metal pipe. Some breakage and chipping was experienced when this was done.

It was found that pyrex tubing could be satisfactorily cleaned in an assembled position.

A bacteriological study of glass tubing cleaned and sterilized without disassembling gave results, from a sanitary standpoint, which were satisfactory and comparable to those obtained when metal pipes are dismantled daily and cleaned in the usual manner practical in the dairy industry at present.

The method used for daily cleaning of the glass tubing in the assembled position was as follows:

Rinse milk lines by circulating (1) cold water, (2) a 0.6% solution of an alkali cleanser containing 4.0% of a wetting agent at a temperature not less than 110° F. for at least 20 minutes, (3) clean water rinse at 110° F., (4) hot water rinse at about 190° F. for not less than 15 minutes. Just before use circulate a chlorine rinse solution of at least 100 ppm. strength through the entire milk processing system. Glass tubing and tube joints opened and examined at intervals of two to six weeks using this treatment were found to be in an excellent sanitary condition.

The optimum time interval for disassembling and examining tubing and joints was not determined. It was suggested, however, that the interval should not be greater than two weeks, until further data were secured.

L.H.B.

54. **Comparative Educational Background of Dairy Graduates, Sanitary Engineers and Veterinarians in Milk Control.** SIDNEY SHEPARD, Birmingham, Ala. Jour. Milk Technol., 6, No. 4: 235. July-Aug., 1943.

"Since World War I the field of dairy science as it relates to public health has slowly but surely drifted from the dairy college graduates into the hands of sanitary engineers (a division of civil engineering) and veterinarians. Today, only sanitary engineers may hold commissions as milk sanitarians with the United States Public Health Service, and only veterinarians are deemed qualified to exercise sanitary supervision over the

production and manufacture of all products of 'bovine origin' being used by our armed forces."

From a study made of the curricula of five dairy colleges, four veterinary colleges and five schools of sanitary engineering ("In each instance the colleges studied were those generally accepted as outstanding in their particular field."), it was obvious that the dairy colleges were doing a good job in educating men for a career in milk sanitation. In every instance they had courses in dairy manufacture, animal husbandry, bacteriology, dairy bacteriology, chemistry, dairy chemistry and agricultural economics.

Of the veterinary colleges studied only two offered any courses in the fundamentals of milk and its products and these were of a limited nature. More courses were offered in animal husbandry, but this was due to the fact that farm animals other than cows were studied.

None of the schools of sanitary engineering offered any courses of study in either dairy manufactures or animal husbandry.

Thus, it would seem that the dairy college graduate would be the logical choice for milk sanitation work.

From a questionnaire sent to cities of more than 100,000 population, it was ascertained that in 32 out of 79 of these cities answering the questionnaire, no dairy graduates were employed in milk sanitation.

The fact that dairy graduates are being discriminated against is probably due to the fact that the dairy colleges have been too prone to overlook the public health field, concentrating their interest in turning out men for the industry; also, many health officials have become oblivious to the virtues of the technically trained dairy college graduate when milk sanitarians are being sought.

"While many milk sanitarians are sanitary engineers who by practice and experience have become expert in this particular line of endeavor, the preeminence of the educational background of the dairy graduate cannot be denied—by this virtue above all, is he (the dairy college graduate) the logical candidate for milk sanitation and all it implies." L.H.B.

55. Properties of Detergent Solutions. Thermal pH Coefficients of Alkaline Solutions. LESTER E. KUENTZEL, JAMES W. HENSLEY, AND LESLIE R. BACON, Wyandotte Chemicals Corp., Wyandotte, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., 35, No. 12: 1286. Dec., 1943.

This is the fourth of a series of papers concerned with the properties of detergent solutions, especially those used in laundry practice. Detailed pH data at 25°, 40°, and 60° C. are presented for distilled water solutions of nine commercial alkalies sometimes used alone and in combination as soap builders. The alkalies examined were sodium hydroxide, sodium carbonate, sodium bicarbonate, trisodium phosphate, tetrasodium pyrophosphate,

sodium tetrphosphate, sodium metasilicate, sodium sesquisilicate and sodium orthosilicate. B.H.W.

56. **Mixed Calcium Salts of Soaps and Anionic Detergents.** GILBERT D. MILES AND JOHN ROSS, Colgate-Palmolive-Peet Co., Jersey City, N. J. Jour. Indus. and Engin. Chem., Indus. Ed., 35, No. 12: 1298. Dec., 1943.

Mixed salts of calcium with fatty acids and synthetic anionic detergents were found to form when mixed in solution. Study of formation of the salts in mixtures containing sulfated detergents, soap and calcium salt permitted appraisal of decrease in foaming and deterative properties of the solutions. No corresponding behavior was found for magnesium salts.

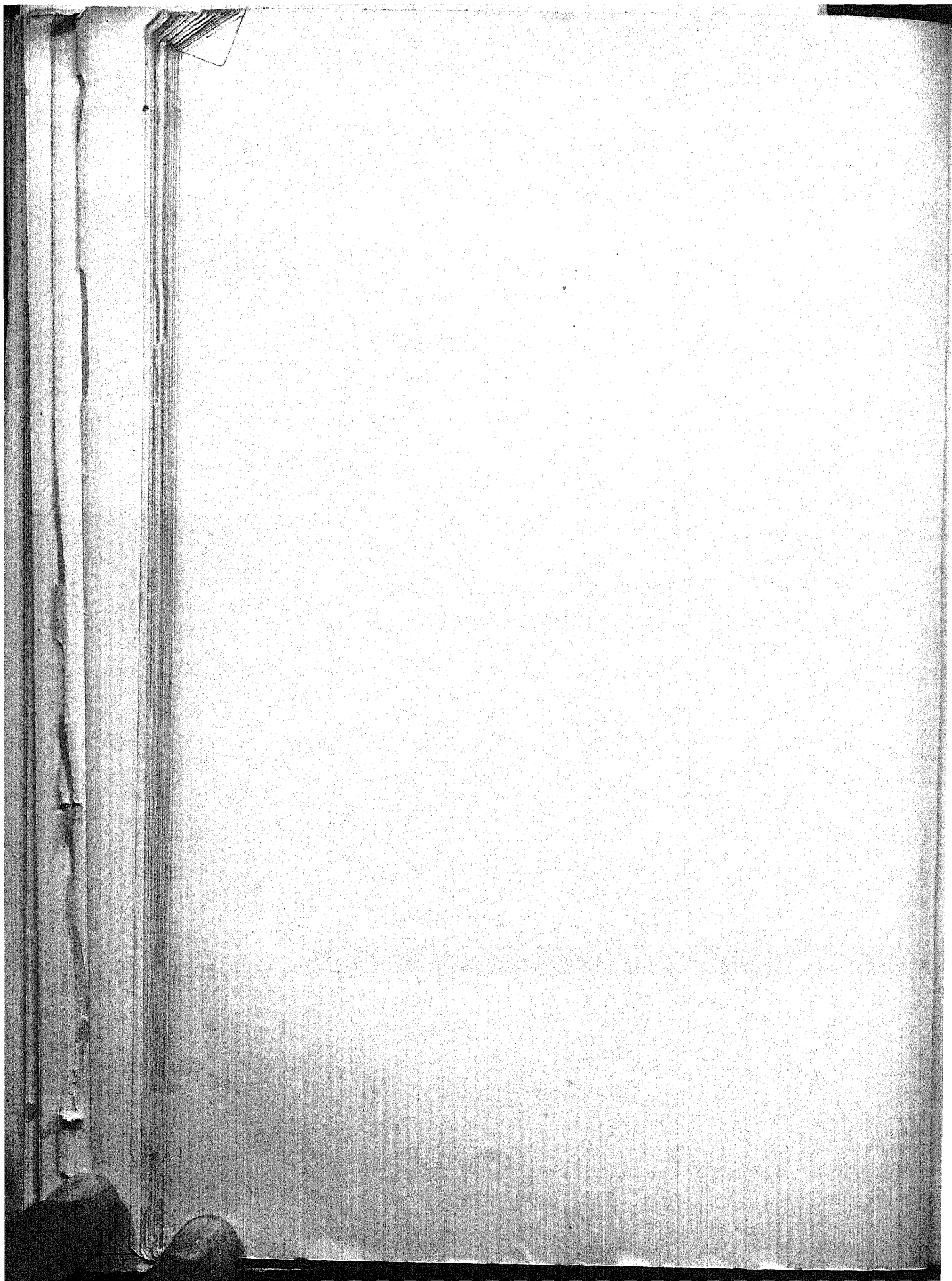
B.H.W.

57. **Labor Saving Through Farm Job Analysis. I. Dairy Barn Chores.** R. M. CARTER. Vt. Agr. Expt. Sta. Bul. 503. 66 pages, illus. June, 1943.

A detailed record was made of the time taken, the distance walked, and the routes traveled by the owner in doing the barn chores for his 22-cow dairy. After careful study of the problem a series of changes, designed to make the work easier and to save time, were made. These changes were of four general types: (1) Rearrangement of the stable; (2) Improvement of work routines; (3) Provisions of adequate and suitable equipment; (4) Convenient location of tools and supplies. As a result, the time spent on chores was reduced from 5 hours 44 minutes to 3 hours and 39 minutes daily, a saving of 2 hours 5 minutes, and the travel was reduced from $3\frac{1}{4}$ to $1\frac{1}{4}$ miles daily, a saving of 2 miles. Two hours a day is equivalent to more than 60 12-hour days, a good 2 months work, in a year; 2 miles daily is equivalent to 730 miles yearly. The money cost of the changes made was small. What this man did can be done by any dairy farmer who will undertake the task seriously. Many of the ideas worked out on his farm can be applied on other farms without change. In other cases some modification may be necessary to make them workable. In still others new schemes may need to be devised to solve particular problems. But the method here used of observing the chores, studying the problem, and working out improvements can be applied anywhere. Specific suggestions for the job are given; also a score card for dairy barn layout.

38 figures, 22 tables.

J.G.A.



ABSTRACTS OF LITERATURE

BACTERIOLOGY

58. **Significance of Adequate Controls in Absolute Sterility Determinations.** D. C. FOORD, C. L. CRANE, AND B. S. CLARK, Res. Dept., Amer. Can Co., San Francisco, Calif. Food Res., 8, No. 6: 489. Nov.-Dec., 1943.

Data accumulated in routine bacteriological examination of paper milk containers over a period of three and one-half years (30,718 tests) by the rinse test of the A.P.H.A. Standard Methods, indicated 78.5% were sterile. If a correction for technique error is made, 97% of the containers were sterile. The non-sterile containers showed fewer than three bacteria per container or approximately 0.003 of the limit permitted by the U. S. Public Health Service Milk Ordinance and Code.

Data are included which demonstrate that the method of incubating nutrient broth in the container, as suggested in Standard Methods, yields an inaccurate measure of the organisms which the container would contribute to milk placed therein, because the container is subjected to conditions which it is not required to meet in commercial use.

Control data for the rinse test indicate that in testing for absolute sterility of any type of containers no less than one control should be made for each three containers tested.

F.J.D.

59. **Extracts from Irish Moss as a Substitute for Agar in Bacteriological Culture Media.** A. W. WALKER AND A. A. DAY, Dept. of Bact., Northwestern Univ., Medical School, Chicago. Food Res., 8, No. 6: 435. Nov.-Dec., 1943.

The possibility of using the gelatinous extract of *Chondrus crispus* (Irish Moss) as a substitute for agar, which is becoming a "critical material," was investigated. The particular product used was "carrageen" made by the Krim-Ko Co. of Chicago, Illinois, to which the manufacturers have given the name "Carragar" when used as a substitute for agar.

Results indicated that "Carragar" can be used as a solidifying agent in bacteriological culture media. The gel is not as firm as agar, the melting point is lower and it is more easily hydrolyzed. However, it is satisfactory for most purposes and can be made firmer by additions of agar to it. Neutralization of the "Carragar" before heating prevents its hydrolyzation.

"Carragar" produced comparable results, in all of the media employed in the study, to those obtained with agar.

F.J.D.

60. A Comparative Study of One Per Cent and Five Per Cent Solutions of 30 to 40 Mesh Gelatins for Bacteriological Examination. TECH. COM. OF THE EDIBLE GELATIN MFGRS., Res. Soc. of America. Food Res., 8, No. 6: 429. Nov.-Dec., 1943.

One per cent solutions of 30- to 40-mesh gelatin are preferable to five per cent solutions for bacteriological examination, in the judgment of the committee. While no results were obtained with coarsely ground or flake gelatins, it is suggested that five or ten per cent solutions will probably yield more reliable results.

F.J.D.

61. Nutritional Studies on *Streptococcus Lactis*. I. An Unidentified Growth Factor Found in Yeast Extract. F. R. SMITH, Univ. of Calif., Davis. Jour. Bact., 46, No. 4: 369. Oct., 1943.

The author describes the preparation and some characteristics of an unknown substance in yeast extract that is essential for the growth of certain strains of *Streptococcus lactis*. This growth factor is apparently not a known vitamin and could not be replaced by a combination of amino acids.

D.P.G.

62. Heat Resistant Organisms in Milk Supplies. W. D. DOTTERER, Dir. of Labs., Bowman Dairy Co., Chicago, Ill. Jour. Milk Technol., 6, No. 5: 269. Sept.-Oct., 1943.

It is quite common to find a great many milk supplies containing thermoduric bacteria. Some areas are a greater source of trouble from these organisms than others.

Utensils have generally been the source, with milking machines involved more frequently than others; however, the cow's udder may also be the source.

High-temperature short-time pasteurization will generally give higher counts than the vat system of pasteurization.

Great care in the cleansing and sterilizing of utensils is necessary for the elimination of thermoduric organisms from milk supplies.

L.H.B.

BREEDING

63. Inhibition of Sperm Glycolysis and Reversibility of the Effects of Metabolic Inhibitors. HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., 148, No. 2: 343. 1943.

Using a 0.02 M glucose solution as a substrate for washed bull spermatozoa the following inhibited glycolysis and their effect was reversible: cyanide, quinone, fluoride and azide; while maleate, hydroquinone, iodo-

acetate and glyceraldehyde inhibited glycolysis but their effect was not reversible.

"In yolk-buffer some specimens of bull spermatazoa could be almost completely inactivated by fluoride for several days and upon transfer to fresh yolk-buffer vigorous motility was regained."

A.O.C.

64. **Inhibition of Sperm Respiration and Reversibility of the Effects of Metabolic Inhibitors.** HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., 148, No. 2: 333. 1943.

The effect of 17 different metabolic inhibitors on the respiration of bull spermatazoa and the reversibility of the effect of these inhibitors is given. Cyanide, malonate and benzoate inhibit the respiration but not the glycolysis of the sperm and are toxic to motility only when glucose is absent. This is further evidence of the ability of 2 separate metabolic processes, *i.e.*, oxidative and glycolytic, to furnish energy for motility of bull sperm. A.O.C.

BUTTER

65. **There Will Always Be Quality.** R. E. ELDERED, The Great Atlantic & Pacific Tea Co., Chicago. Natl. Butter and Cheese Jour., 35, No. 1: 8. Jan., 1944.

The customer today wants quantity rather than quality. Inferior cream and butter command the same prices as better products. Some factors causing quality deterioration are: delays in cream pick-ups, farm labor shortage, relative prices for farm and dairy products, curtailment of educational field work, discrepancies in official gradings, lack of food regulatory personnel, increased production of unsalted butter and differences of point values in farm and creamery butter. But quality has not been forgotten because critical customers are asking for better butter and the army, which is buying good table butter, is educating the tastes of more men. As normal trading conditions are resumed receivers will be more strict, the Food and Drug departments will again do routine checking and the natural pride of butter makers will all tend to improve quality. Large milk drying operations will produce fine cream, some of which will be used for butter, and small operators may have to develop cooperative field services to compete with these larger concentrators.

W.V.P.

66. **Looking into the Future of Creamery Operations.** H. E. BEHLMER, Cherry-Burrell Corp., Chicago. Natl. Butter and Cheese Jour., 34, No. 12: 18. Dec., 1943; and 35, No. 1: 36. Jan., 1944.

Future efforts to improve quality and lower manufacturing costs will probably require changes in methods and machinery. Cream quality will

be improved by education of producers, development of better-equipped cream receiving units, and more efficient utilization of milk-solids-not-fat. Innovations in creameries will include: continuous butter manufacture without churns, more general use of the vacuum-type pasteurizer, better cooling practices, and coolers designed to control temperatures and deliver cream to churns instead of holding tanks. Other significant changes expected are: greater use of stainless steel in vats, pipes, pumps and refrigerating units, better sanitation, more general use of storage tanks instead of coil vats and the use of better lubricants.

W.V.P.

67. **Determination and Content of Carotene and Vitamin A in Wisconsin Butter.** S. BERL AND W. H. PETERSEN, Dept. Biochem., Univ. Wis., Madison. Jour. Nutr., 26, No. 5: 527-538. Nov., 1943.

A solvent extraction is described for the determination of carotene and vitamin A in butters, utilizing the yellow color of the carotene for its determination and the Carr-Price reaction for the determination of vitamin A.

Diacetone alcohol, 94%, proved to be superior to 92% methanol for the extraction of non-carotene pigments.

Twenty-two samples of butter made during the week of March 23, 1942, by creameries in southwestern Wisconsin, and 20 samples of butter from the same creameries in July, 1942; and samples of butter made in September and January collected from various sections of Wisconsin were used in this study.

The March butters averaged 9,500 I.U.; the July and September butters 18,000 I.U.; and the January butters 10,500 I.U. per pound. In the summer butters about 75% of the total natural butter pigment was found to be carotene, in the winter butters 60 to 65% of pigment was carotene.

Storage of butter at -22 to -23° F. as long as 8 months did not result in a loss of carotene or of vitamin A.

C.F.H.

68. **The Iron Content of Butter and Its Relation to the Butter Wash Water.** J. B. LINNEBOE, Alberta Dept. Agr., Edmonton. Sci. Agr., 24, No. 2: 64. 1943.

Samples of butter from 146 churnings representing 27 creameries were analyzed for iron. The iron content of the wash water from 26 of these creameries were also determined.

The iron content of the butter ranged from 0.4 ppm. to 3.3 ppm. The butter possessing the lower iron content tended to grade higher and there was also a tendency towards uniformity in the iron content of the butter from the same creamery, particularly the butter of first grade.

The iron content of the wash water was shown to affect the iron content of the butter. Where portions of churnings were removed from the churn and washed with distilled water or natural water, low in iron, all the first grade butter contained less than 1.0 ppm. of iron.

O.R.I.

CHEESE

69. Cottage Cheese from Soybean Curd? ANONYMOUS. Milk Dealer, 33, No. 3: 27-56. Dec., 1943.

Following a description of making cottage cheese from soybeans' milk, as released by Science Service, a survey of the possibilities of making a substitute cottage cheese by dairy plants is made. Methods of treating soybean milk to produce a curd comparable in many respects to regular cottage cheese made from skimmilk have been to culture milk with lacto-bacillus or soy acidophilus organism; to use an alkali (magnesium chloride) to coagulate the soybean milk or to curdle the milk with a weak acid. A patent for manufacturing has been applied for by one dairy plant. The possibility of providing a substitute cottage cheese under existing shortages of skimmilk, which possesses some of the texture, flavor, acidity and moisture content characteristics of regular cottage cheese is discussed. C.S.T.

70. Starters for Cheese Making. G. M. MOIR, Dairy Chemist. New Zeal. Dept. Agr. Bul. No. 162.

This bulletin brings together the more important research and practical observations on the making of starters as applied to cheese factory conditions in New Zealand.

Starters are used to ripen the milk before renneting, to secure proper acidity during the making process, to restrain undesirable bacteria, and to aid in cheese ripening. The starter must contain the right bacteria, and no others, in active condition capable of developing acidity at the proper rate. The cultures in New Zealand are often pure strains of *Str. cremoris*, but usually *Str. lactis* and *Str. citrovorus* are also present.

Bacteriophage is a common cause of limited acid development. The phage infection usually occurs from the "whey frog" from a whey separator, from dust, or from contamination from phage-infected starters. Some other conditions that cause slow acid development are low temperatures of incubation, growth-retarding substances in the milk, aeration of the milk after pasteurization, and too small inoculation, but phage contamination is most important.

The objection to excessive acidity (1.0-1.2%) in former years was due to poor starters. Actually 0.65-0.85% is quite satisfactory and overripening is no longer so common due to use of proper bacteria.

Special milk should be selected for starters. Special starter rooms in cheese factories are desirable. Several starters are preferred to one to assure against complete failure. Mother cultures are best carried in glass containers. Stress is given to cotton plugging the openings for inoculation for the main and mother starter and of flaming these openings when transfers are made to avoid phage contamination. Details of making the starters are given and the Whitehead and Cox vitality test is described.

It is pointed out that phages have not been troublesome in America and that mixed cultures are probably preferable to single strains to control phage difficulties.

A.C.D.

CHEMISTRY

71. **Modifications of the Swift Stability Test.** R. W. RIEMENSCHNEIDER, J. TURER, AND R. M. SPECK, Eastern Regional Res. Lab., U.S.D.A., Philadelphia, Pa. *Oil and Soap*, 20, No. 9: 169-171. Sept., 1943.

An all-glass aeration tube for use in the accelerated method of determining stability of fats was found to have several advantages over the rubber-stoppered test tubes. An improved air-distributing apparatus is also described. The capacity of the apparatus has been increased three-fold by the adoption of a procedure which permitted the use of only one tube for each test sample.

Factors influencing the peroxide values as determined by adaptation of Wheeler's method were investigated.

J.L.H.

72. **The Development of a Practical Antioxidant for Lard and Shortening.** A. LIPS AND W. D. MCFARLANE, Dept. of Chem., Macdonald College (McGill Univ.), Ste. Anne de Bellevue, Quebec, Canada. *Oil and Soap*, 20, No. 10: 193-196. Oct., 1943.

Wheat germ oil, extracted by means of ethylene dichloride and fortified with citric acid, was found to be an efficient antioxidant in lard and shortening. The wheat germ oil supplied the phenolic type of antioxidant in the form of tocopherols and the citric acid, the acidic component necessary in an antioxidant intended for use in both animal and vegetable fats and oils.

J.L.H.

73. **The Solubility of Gases in Butter Oil, Cottonseed Oil, and Lard.** P. S. SCHAFER AND H. S. HALLER, Bur. of Dairy Indus., U. S. D. A., Washington, D. C. *Oil and Soap*, 20, No. 8: 161-162. Aug., 1943.

The solubility of hydrogen, oxygen, air, nitrogen, and carbon dioxide at 40° C. is slightly higher in butter oil than in cottonseed oil and lard. The solubilities of the above gases in butter oil at 40° C. and 60° C. are shown in the following table:

Gas	Gas dissolved in 100 ml. butter oil	
	40° C.	60° C.
	ml.	ml.
Oxygen	14.2	12.7
Nitrogen	8.9	7.9
Hydrogen	5.4	6.8
Air	10.1	9.6
Carbon dioxide	109.5	91.0

J.L.H.

74. **The Application of the Ferric Thiocyanate Method to the Determination of Incipient Rancidity in Fats and Oils.** A. LIPS, R. A. CHAPMAN, AND W. D. MCFARLANE, Dept. of Chem., Macdonald College (McGill Univ.), Ste. Anne de Bellevue, Quebec, Canada. *Oil and Soap*, 20, No. 11: 240-241. Nov., 1943.

A colorimetric method for determining fat-peroxides in whole milk powder, previously reported by the authors, has been modified for use in fats and oils. The method is based on the oxidation of ferrous to ferric iron by the peroxides present in oxidized fats. The ferric iron is determined as ferric thiocyanate; the intensity of the color being measured with a Coleman spectrophotometer set at 485 m μ . J.L.H.

75. **A Rapid Test for Alpha Dicarboxyls.** L. O'DANIEL AND L. B. PARSONS, Res. Dept., Lever Bros. Co., Cambridge, Mass. *Oil and Soap*, 20, No. 4: 72-74. Apr., 1943.

When alcoholic caustic potash is added to auto-oxidized fats and oils more or less highly-colored solutions are formed, the color depending upon the type of fat and the extent of oxidation. The color is probably due to quinoid compounds formed by aldol condensation of alpha-diketones in a manner analogous to the formation of para-xyloquinone from diacetyl. Linoleic and possibly linolenic acids or esters are undoubtedly the source of the quinoid compounds. A rapid test for alpha dicarboxyls in fats and oils is described. J.L.H.

76. **The Antioxidative Behavior of Vegetable and Animal Fats.** CALVIN GOLUMBIC, Bio-Chem. Lab., State Univ. of Iowa, Iowa City. *Oil and Soap*, 20, No. 6: 105-107. June, 1943.

Autoxidizing animal fats exhibit a well-defined induction period, the end of which coincides with the development of oxidative rancidity. Vegetable fats, however, do not have a sharply defined induction period and they show oxidative rancidity before the period of relatively rapid oxygen uptake and peroxide formation.

A kinetic study of the oxidation of tocopherol during the induction periods of animal and vegetable fats yielded information on possible causes of the differences in the induction periods. The oxidation of tocopherol in both fats is accompanied, in the early stages, by the formation of tocoquinones. In animal fats the complete disappearance of added tocopherol marks the end of the induction period and the beginning of organoleptic oxidative rancidity.

Chroman, -5, 6-quinones appear during the course of the induction period of autoxidizing vegetable, but not animal fats. These O-quinones, derived from unknown precursors, are antioxidants and their relatively slow oxidation rate as compared with tocopherol permit them to act after the

disappearance of tocopherol. This action offers an explanation for the absence of sharp induction periods of vegetable fats.

Tocopherols are decreasingly effective as antioxidants when employed at higher levels. This accounts for the previously recognized ineffectiveness of tocopherols and inhibitol concentrates when added to vegetable fats.

J.L.H.

77. **Estimation of Vitamin A in Food Products.** BERNARD L. OSER, DANIEL MELNICK, AND MORTON PADER, Food Res. Labs., Inc., Long Island City, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., 15, No. 12: 724. Dec., 1943.

Modification of the antimony trichloride method for determination of vitamin A in food products, including dairy products, was made. Corrections are allowed for the presence of inhibitors of the color development, for temperature effects, for variations in the reagent, for turbidities produced in the course of the color development, and for extraneous color present in the final test solution. The unreliability of the direct spectrophotometric method for the assay of foods is demonstrated. The reaction of carotene with antimony trichloride was studied.

B.H.W.

78. **Factors Affecting the Stability of Cottonseed Oil. A Study of the Antioxygenic Activity of Alpha-Tocopherol.** C. E. SWIFT, W. G. ROSE, AND G. S. JAMIESON, Bur. of Agr. Chem. and Engin., U.S.D.A., Washington, D. C. Oil and Soap, 19, No. 10: 176. 1942.

The results secured suggest that the antioxygenic activity of the tocopherols is due to their reactivity towards the active peroxides. The rate and extent of peroxide accumulation during the induction period was found to be dependent on the tocopherol content of the oil. The tocopherols function most effectively at lower levels of concentration and with decreasing efficiency at higher levels. Small amounts of the cephalin fraction markedly retarded the rapid initial rate of oxidation of α -tocopherols. This effect demonstrates the synergism of an "acid-type" substance acting with a "phenolic-type" antioxidant.

J.L.H.

79. **The Oven and Aeration Methods as Means of Accelerating Fat Oxidation.** F. C. EWBANK AND I. A. GOULD, Dept. of Dairying, Mich. State Col., East Lansing, Mich. Oil and Soap, 19, No. 12: 205. 1942.

A comparison was made of the aeration and hot oven methods of accelerating butter oil oxidation at 100° C. The rate of peroxide formation was used in determining the induction period; a peroxide value of 5 being arbitrarily chosen as the end of the induction period. The two methods were

found to be equally reliable when careful technique was employed and where the influence of certain variable factors were controlled. With the oven method especial attention must be given to temperature control, air agitation and arrangement of the samples in the oven if uniform results are to be obtained. The oven method gave a shorter induction period than the aeration method but the extent of oxidation over a relatively long period was less.

J.L.H.

80. **The Antioxygenic Action of Phosphoric Acid in Association with Tocopherols and Hydroquinones.** CALVIN GOLUMBIC, Bio-Chem. Lab., State Univ. of Iowa, Iowa City, Ia. *Oil and Soap*, 19, No. 10: 181. 1942.

Phosphoric acid was found to retard the oxidation of tocopherol in autoxidizing fats and thus to increase its antioxygenic activity. The stabilizing capacity of hydroquinones was likewise found to be increased by small amounts of phosphoric acid. The data secured suggest that this synergistic action is due to the shifting to the left, of the hydroquinone \rightleftharpoons quinone equilibrium. The tocophydroquinone \rightleftharpoons tocoquinone equilibrium is a special case of this relationship in which phosphoric acid also catalyzes the cyclization of tocophydroquinone to tocopherol, thus regenerating the antioxidant.

J.L.H.

81. **Vitamin A Added to Fats as Related to Stability During Baking.** E. E. RICE, H. C. BLACK, G. T. CARLIN, AND H. E. ROBINSON. Swift & Co., Chicago, Ill. *Oil and Soap*, 19, No. 9: 164. 1942.

The Carr-Price color reaction and U.S.P. bio-assays were used to determine the stability of vitamin A in various types of baked goods prepared with fats fortified with vitamin A. In bread, biscuits and cake which are relatively low in fat and baked under moderate conditions it appears that 80 to 100% of the vitamin survives the baking process. When the fat content is higher and the baking conditions more severe as in pie crust, considerable vitamin A destruction is likely to occur depending on the extent of the baking.

J.L.H.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

82. **The Nitrogen Distribution in Dried Milk.** U. S. ASHWORTH AND HARRIS O. VANORDEN, Div. of Dairy Hus., Agr. Expt. Sta., Pullman, Wash. *Jour. Milk Technol.*, 6, No. 5: 272. Sept.-Oct., 1943.

Samples of spray-dried skimmilk were analyzed to determine whether the nitrogen distribution differed from that of normal skimmilk.

The results obtained when calculated to a moisture-free basis were found to agree very well with those reported in the literature for fresh skimmilk

with the exception of the albumin-plus-globulin fraction. Out of 32 samples tested only four contained measurable amounts of this fraction. The amount found, no doubt, was associated with the heat treatment that the samples received prior to drying. L.H.B.

83. **A Laboratory Spray Drier.** A. H. WOODCOCK AND H. TESSIER, Natl. Res. Labs., Ottawa. *Canad. Jour. Res., A*, 21, No. 9: 75. 1943.

A laboratory model of a cyclone-type spray drier which in operation has produced dried milk and dried egg of good commercial quality is described. The main drying chamber is an inverted cone 2 feet in diameter and 5 ft. 2 in. in height made of galvanized metal and covered with insulating materials. Adjustments are provided for inlet air temperature, total air flow, angular velocity of air in the drying chamber, quantity of liquid introduced, and spraying technique. The air is supplied by a fan similar to the type used in a large household vacuum cleaner and is heated by a thermostatically controlled, 5000-watt electric element. The maximum capacity is about four liters of liquid per hour. O.R.I.

DISEASE

84. **Newer Methods for the Control of Bovine Mastitis.** G. R. SPENCER, Dept. of Vet. Sci., Univ. Wis. *Milk Dealer*, 33, No. 1: 52. Oct., 1943.

To control the spread of mastitis by the dairyman, the infected animals must be "spotted" "by the strip cup and the Hotis test." Both tests are needed to determine both new or "carrier" cows. Segregation is recommended in lieu of slaughter at the present time as a means of preventing spread of infection. Segregated animals should be milked and handled by special personnel if possible, and if not, every sanitary precaution exercised to prevent spread of infection such as reduced feeding to facilitate treatment; thorough milking of all quarters; avoidance of teat injuries and specific medicinal treatment to cure and reduce extent of infection, are recommended as control measures. C.S.T.

85. **Production and Prevention of Bloat in Cattle on Alfalfa Pasture.** H. H. COLE, S. W. MEAD, AND W. M. REGAN, Univ. Calif., Davis, Calif. *Jour. Anim. Sci.*, 2, No. 4: 285-294. Nov., 1943.

Bloat was produced at will in dairy cows by depriving them of hay and bedding for at least 48 hours and pastured on thick stands of immature alfalfa that was 8 to 14 inches high. A thick stand makes possible rapid ingestion. It was necessary to use pastures free from weeds and contaminating grasses. Cows tend to select considerable amounts of coarse, seemingly unpalatable weeds where succulent alfalfa is abundant.

The availability of the water in the pasture did not affect the incidence of bloat. The feeding of alfalfa hay reduced the incidence and severity of bloat but was not completely effective. Bloat was effectively controlled on alfalfa pasture by feeding Sudan hay in the corral or in the pasture. Also, pasturing on Sudan at night before placing the cows on alfalfa pasture appeared to be an effective means of controlling bloat. Cows ruminated only one-half as much on alfalfa pasture as on Sudan pasture. Apparently both rumination and belching are readily induced by Sudan because of scabrous leaves. C.F.H.

86. **A Cheese-Borne Epidemic of Typhoid Fever.** JACQUES GAUTHIER AND A. R. FOLEY, Min. of Health and Soc. Welfare, Quebec. *Canad. Jour. Pub. Health*, 34, No. 12: 543. 1943.

An outbreak of typhoid fever which resulted in 40 cases and six deaths is described. Evidence indicated that milk supplied to the cheese factory became contaminated from a carrier working on a farm. The cheese was of the Cheddar type and was consumed when about ten days old. Butter made in the same factory from pasteurized cream was not incriminated. In addition to the control measures taken the following recommendations are made:

1. Early notification of each case of enteric fever.
2. General check-up of typhoid carriers.
3. Pasteurization of the cheese milk or at least the holding of the product for a three-month period before consumption. O.R.I.

87. **Effects of Disease on Nutrition. I. Absorption, Storage, and Utilization of Vitamin A in the Presence of Disease.** SAMUEL SPECTOR, CHARLES F. MCKHANN, AND EMILY R. MESERVE, Dept. of Ped. and Communicable Dis., Univ. of Mich. Med. School, Ann Arbor, Mich. *Amer. Jour. Dis. Children*, 66, No. 4: 376-395. Oct., 1943.

This paper is a review of the effects of disease on absorption and utilization of vitamin A. Most of the data cited were obtained with human subjects, and the review has been made from the viewpoint of human medicine. The effects of several pathological conditions are discussed, which includes jaundice, celiac syndrome, cystic fibrosis of the pancreas, infection, cirrhosis of the liver, valvular lesions of the heart, malignant neoplastic tissue, disorders of the thyroid, and allergy.

It is pointed out that malnutrition may be the result of organic disturbances within the patient. For example, during infections there develops rather regularly a decrease in the ability of the intestinal tract to absorb carotene and vitamin A. This decreased absorptive power occurs even with infections that do not primarily involve the gastrointestinal tract.

It is not possible to make a complete review of this article, as it in itself is a review. Sixty-two references are given. R.K.W.

FEEDS AND FEEDING

88. Feeding Standard Equation for Cows and Goats in Milk. W. L. GAINS, Univ. Ill., Urbana, Ill. Jour. Anim. Sci., 2, No. 4: 304-313. Nov., 1943.

The precision data used in this investigation were reported in the literature from the Pennsylvania Agricultural Experiment Station. One hundred seven observations on 10 cows were used in the formulation of a standard equation for lactating cows. The feeding standard equation for the milking cow is as follows:

$DN = 0.008 W + 0.3 \text{ F.C.M.}$, in which DN = daily digestible nutrients intake in pounds, W = live weight in pounds, F.C.M. = daily milk-energy yield in pounds 4% milk. C.F.H.

89. Studies on the Alimentary Tract of Merino Sheep in South Africa. VI. The Role of Infusoria in Ruminal Digestion with Some Remarks on Ruminal Bacteria. J. G. VAN DER WATH AND S. J. MYBURGH, Section of Biochem., Onderstepoort, Pretoria, South Africa. Onderstepoort Jour. Vet. Sci. and Anim. Indus., 17, Nos. 1 & 2: 61-85. Oct., 1941.

A technique is described for the preservation and counting of ruminal infusoria.

The changing of the ration of sheep from alfalfa and corn to corn alone resulted in extinction of the large type of infusoria and a reduction of all types following the cessation of rumination. Appetite of the sheep was adversely affected. A ration of wheat straw alone resulted in the starvation of ruminal infusoria. The addition of corn to the wheat straw resulted in a marked increase in the number of infusoria. The feeding of green alfalfa only brought about a reduction in the number of infusoria.

Season fluctuations of ruminal infusoria of grazing sheep are described. The amount of protein in pasture had a significant influence on the density of the infusoria population.

The rate of digestion of fine yellow corn meal by starved infusoria in the rumen was studied. Yellow corn particles were found in the infusoria one minute after being placed in the rumen. Disintegration of corn particles was observed within the infusoria. The so-called glycogen granules observed within the plasma of the infusoria were shown to be glycogen-synthesizing bacteria. The food and bacteria ingested by the infusoria were digested by enzymes secreted by the bacteria present. In an experiment where the infusoria and bacteria were destroyed by feeding copper sulphate and the rumen inoculated with starch-digesting bacteria, it was shown that infusoria are not necessary for starch digestion.

Infusoria engulfed particles of cellulose. The digestion of cellulose within the body of the infusorium is primarily due to cellulose-digesting bacteria ingested by the infusorium.
C.F.H.

90. Brown Silage from Atlas Sorgo—Chemical Composition and Apparent Digestibility as Determined by Feeding to Dairy Cows.

H. E. BECHTEL, F. W. ATKESON, AND J. S. HUGHES, Kans. Agr. Expt. Sta., Manhattan, Kans. Jour. Anim. Sci., 2, No. 4: 295-303. Nov., 1943.

Chemical analyses of five brown silage samples and one sample of high moisture chopped brown fodder were compared with those of normal green Atlas sorgo silage. Three silages were used in digestion trials. (1) Normal green silage from a wire and roofing paper silo in which maximum temperature during storage was 92° F. (2) Brown silage from sorgo bundle silage in which the maximum temperature during storage was 141° F. (3) Brown silage from a straw bale silo in which the maximum temperature during storage was 147.5° F.

The chemical analyses showed no appreciable difference between the brown and normal silages except for carotene and possible ash. The amount of carotene in the brown (high temperature) silages was very low.

There was a marked decrease in the coefficients of digestibility for all nutrients except ether extract in the brown silage. The degree of digestibility appeared to depend on the maximum temperature during storage. Protein was most affected, the apparent digestibility averaged 55% for normal silage, 23 and 4% for the two brown silages.

Brown silage was less palatable than normal silage.

C.F.H.

91. Biological Methods of Measuring the Protein Values of Feeds. H. H. MITCHELL, Div. Anim. Nutr., Univ. Ill., Urbana, Ill. Jour. Anim. Sci., 2, No. 4: 263-277. Nov., 1943.

The evaluation of biological methods of measuring the protein values of feeds is discussed. Conditions considered essential in measuring protein values of feeds are as follows:

1. The measurement of protein utilization by studying the nitrogen economy of the animal.

2. The measurement selected must involve the use of dietary protein in maintenance as well as in production.

3. The imposition of comparable levels of protein feeding.

4. The control of the food intake of comparable animals, which generally means the equalization of food consumption.

C.F.H.

92. Feeding Dairy Stock in Wartime. H. S. WILLARD. Wyo. Agr. Expt. Sta. Bul. 263, 19 pages.

Although an experiment station publication this is primarily an exten-

sion bulletin. Information is furnished on the use and importance of hay and pasture in dairy production, correct apportionment of grain allowances under differing conditions, grain mixtures suitable for feeding with different kinds of roughage, and wartime production of dairy calves and heifers. Several tables are included for the guidance of the stockman. J.G.A.

93. **The Influence of Dietary Fat of Varying Unsaturation on the Component Acids of Cow Milk Fats.** T. P. HILDITCH, Dept. of Indus. Chem., Univ. of Liverpool, AND H. JASPERSON, Res. Dept., Messrs. J. Bibby and Sons, Ltd., Liverpool. *Biochem. Jour.*, 37, No. 2: 238-243. 1943.

In most feeding trials wherein different oils or fats have been fed to dairy cows the results have been interpreted from the over-all or gross change in the degree of saturation of the butter fat produced, but in this particular study an attempt was made to differentiate between the component fatty acids responsible for this gross change.

Fifteen cows were divided into five groups and fed (1) a basal diet; (2) basal diet plus peanut oil which is highly unsaturated, having an I.v. (iodine value or iodine number) of 88; (3) basal diet plus partially hydrogenated peanut oil having approximately the same I.v., 45, as soft butter fat; (4) basal diet plus hydrogenated peanut oil which was almost completely saturated—I.v. of 17; and (5) basal diet plus palm kernel oil which is naturally highly saturated, I.v. of 17.

Groups (2) and (3) produced butter fat with an increased amount of oleo-glycerides and a decrease in the butyric to caprylic glycerides. Group (5) produced butter fat with the lauro-glycerides three-fold greater and myristo-glycerides 20% greater than the control, but the oleo- and palmito-glycerides showed a slight decrease. Group (4) receiving the highly hydrogenated or saturated peanut oil gave butter fat more nearly like the control group in its composition. This is explained on the basis that about half of the fat fed was so completely saturated that its melting point was above the body temperature of the cow and so was not assimilated. It is interesting to note that although the fats fed to groups 4 and 5 had the same I.v. they produced butterfats of quite different detailed fatty acid compositions. Peanut oil normally contains about 20% of the unsaturated acids, linoleic, arachidic, and lignoceric acids, but these did not pass into the butter fat. It seems that the mammary gland has a selective action in the absorption of oleic (as distinct from linoleic) acids.

A good review of previous work is given.

A.O.C.

94. **The Utilization of Urea in the Bovine Rumen.** 1. **Methods of Analysis of the Rumen Ingesta and Preliminary Experiments in Vivo.**

R. M. PEARSON AND J. A. B. SMITH, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. *Biochem. Jour.*, 37, No. 1: 142-148. 1943.

"The relative merits of trichloroacetic acid, sodium tungstate with sulfuric acid, and alcohol have been compared as precipitants in the estimation of non-protein-nitrogen in rumen ingesta. A description is given of the methods finally adopted for the estimation of non-protein-nitrogen, urea and ammonia."

Samples of the rumen ingesta were taken for analysis from the rumen fistula of a steer, but the authors concluded that there were so many difficulties involved in the *in vivo* experiments, especially the fact that it was practically impossible to secure a representative sample, that "the results of *in vivo* experiments of this type cannot be regarded as supplying evidence either for or against the theory that urea is converted to protein in the rumen," and because of this, they state that *in vitro* methods would be more reliable.

A.O.C.

95. The Utilization of Urea in the Bovine Rumen. 2. The Conversion of Urea to Ammonia. R. M. PEARSON AND J. A. B. SMITH, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. *Biochem. Jour.*, 37, No. 1: 148-153. 1943.

Samples of rumen ingesta taken from the gastric fistula of a steer were used in making *in vitro* studies of its action on urea.

All of the urea which would ever be likely to be fed to a cow will be converted to ammonia within an hour.

The urease preparation derived from the rumen was found very similar to the ureases from soya or jack beans in its behavior to temperature, pH and inhibitors. Preliminary attempts to obtain enzyme preparations free from bacteria proved unsuccessful.

A.O.C.

96. The Utilization of Urea in the Bovine Rumen. 3. The Synthesis and Breakdown of Protein in Rumen Ingesta. R. M. PEARSON AND J. A. B. SMITH, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. *Biochem. Jour.*, 37, No. 1: 153-164. 1943.

The liquid rumen contents taken from a gastric fistula of a steer were studied *in vitro*. In order to avoid any appreciable change in the "microbiological picture" incubation periods of only 2 to 4 hours were used. Urea was added to the liquid before incubation. Most all of the non-protein nitrogen was made up of urea and ammonia. Synthesis of protein appeared to occur to the extent of about 9 mg. N/100 gms. rumen liquid. While the total nitrogen remained constant, there was a decrease in the non-protein nitrogen accompanied by a parallel decrease in the ammonia, suggesting that the protein synthesis was from ammonia rather than from urea. This

conversion of ammonia to protein is thought to be microbiological in nature. Accompanying the protein synthesis is a protein breakdown—and either may predominate depending upon the general conditions or the substances present.

A.O.C.

97. Urea as a Partial Substitute in the Feeding of Dairy Cattle. E. C. OWEN, J. A. B. SMITH, AND N. C. WRIGHT, Hannah Dairy Res. Inst., Kirkhill, Ayr, Scotland. *Biochem. Jour.*, 37, No. 1: 44-53. 1943.

Results are given for feeding trials with seven lactating Ayrshire cows over a period varying from 100 to 160 days wherein a third of the nitrogen of the feed was given in the form of urea and then substituted for an equal amount of nitrogen in the form of blood meal.

"The nitrogen balance and excretion data show that, although urea was partially retained by all the animals, its retention was not complete. Compared with blood meal, amounts varying from some 12 to 47% and averaging 25% of the ingested urea apparently passed through the animal without being utilized. This apparent wastage was much reduced when urea feeding was preceded by a period in which the diet had been deficient in total nitrogen.

"The milk yields of five of the seven animals were well maintained when blood meal was replaced by urea. With four of the five cows under test a rapid and significant decrease in milk yield took place when urea was removed from the food."

Data for milk yield, body weight and nitrogen balance are given for the periods. There were no significant differences in the composition of the milk.

A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

98. Dairy Products in the Wartime Dietary. H. H. MITCHELL, Prof. of Anim. Nutr., Univ. of Ill., Urbana, Ill. *Milk Dealer*, 32, No. 10: 28-30, 78-80. July, 1943.

Despite the fact that milk supplies a generous amount of essential nutrients, its value is in the variety of ways it may be used to serve different purposes on man's menu. The processing of milk into the wide variety of products to which it lends itself show the changes even in nutritive value to which it is adapted. As an example, Limburger cheese may increase two to three times in pantothenic acid, niacin and biotin due to the curing process. Fortified oleomargarine and butter are compared nutritionally. The author deplors the advent of the role medical men are taking in formulating nutritional policies to the exclusion of scientific research, since to the medical profession nutrition "is only one of a number of fields of interest" and therefore lead to deductions not subscribed to by trained nutri-

tionists. The fortification of bread with vitamins and the possible exclusion of milk-solids-not-fat is cited as an undesirable procedure since 6% of skim milk powder added to bread "improves the growth-promoting and bone calcifying value of the bread much more than does its enrichment with the official proportions of thiamin, niacin, and iron." Skim milk powder would improve the nutritive value of all bread. The shift to greater sale and use of whole milk from the farm as a wartime need is discussed in its relationship to supplanting skim milk for animal feeding purposes which "would raise serious obstacles in the production of pork, poultry and eggs" were it carried to an extent recommended by some economists. The efficiency of the dairy cow in converting concentrate grain into milk of greater nutritive value than the grain consumed is given as 70% as compared with the 72 to 75% efficiency of the miller converting wheat into patent flour of lower nutrient value than the wheat processed.

C.S.T.

99. **Dairy Research and Human Nutrition.** O. E. REED, Chief of Bur. of Dairy Indus., Agr. Res. Admin., U.S.D.A., Washington, D. C. *Milk Dealer*, 32, No. 12: 35, 78-88. Sept., 1943.

Research in human nutrition is responsible for increased emphasis on the nutritive quality of food supplied to both our armed forces and civilians in this war, whereas quantity of food only was stressed in the last war. Dairy and animal research since the last war has made available information on what is needed to supply human nutritional needs, as to the kind and amount of nutrients required to maintain health, and the effect of lack of certain essential nutrient substances upon physical development. Research and surveys conclusively reveal the high place of milk as prerequisite for nutrition and health in the diet. The United States produces enough milk to provide a quart of milk per day per individual, but unfortunately we do not consume that quart in its entirety but instead utilize only the cream, the butterfat or the curd to the detriment of our nutritional welfare. The author traces the historical growth of the butter and cheese industry and inventions which made possible the complete utilization of the milk as it comes from the cow. The era of vitamins starting in 1912 and 1913 as the result of research gave an impetus to use of all the milk as well as to the respective merits of different vitamins. Nutritional research on vitamin A content of butter was started in 1941 and 23 states are cooperating in this nutritional survey. The "wealth of essential nutrients below the cream line" is likewise being studied. The study of the proteins, milk sugar and inorganic salts of milk but substantiate the importance of the nutritional role such ingredients play in an adequate diet, over and above the role vitamins of milk play. The role of still other constituents of milk wherein a growth factor has been shown is being studied and "every nutritive essential that we have discovered to date has been found to exist in the milk of

the cow. . . ." "Dairy products and good nutrition are inseparable. Good nutrition is fundamental for the physical, economic and moral progress of our people." By marketing the entire output of the dairy cow the dairy industry can best contribute to that progress.

C.S.T.

100. War Needs Teach Nutritional Value of Milk to British Consumers. GEORGE WALWORTH. *Milk Dealer*, 33, No. 3: 32-33. Dec., 1943.

Previous to the war, British fluid milk consumption averaged only one-third pint per day per capita, and efforts to subsidize milk for school children and general use largely failed. With the advent of war, however, the need for adequate nutrition and the recognition of milk as a basic food to prevent malnutrition of mothers, children and low-income groups was recognized by the British Government, and its distribution to such groups was subsidized and rationed on a preferential basis. The postwar effect of the recognition of the valuable nutritional role of milk and dairy products to the British consuming public should result in increased sale of all such products and serve as "the finest investment a government can make for the health and welfare of its people."

C.S.T.

101. The Ratio of Ascorbic Acid, Riboflavin and Thiamine in Raw and Pasteurized Milk. A. D. HOLMES, C. P. JONES, A. W. WERTZ, AND J. W. KUZMESKI, *Mass. Agr. Expt. Sta., Amherst, Mass. Jour. Nutr.*, 26, No. 4: 337-345. Oct., 1943.

Composite milk from a herd consisting of 18 Ayrshires, 13 Guernseys, 18 Holsteins and 11 Jerseys, collected during January, February and March was used in this study. The milk was held at 40° F. for an average of 10 hours prior to pasteurization in a stainless steel vat, pasteurized by the holding process for 30 minutes at 143-145° F.

The ascorbic acid content of the raw milk ranged from 14.0 mg. to 22.5 mg. and averaged 19.7 mg. per liter; after pasteurization, the extreme values were 7.0 mg. and 19.1 mg., with an average value of 15.9 mg. per liter. The loss of ascorbic acid during pasteurization was 18.3%.

The riboflavin content of the raw milk varied from 1.35 mg. to 1.75 mg. and averaged 1.51 mg. per liter. The corresponding riboflavin values for the pasteurized milk were 1.19, 2.06, and 1.48 mg. per liter, respectively. The loss of riboflavin during pasteurization was only 2%.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. and averaged 0.33 mg. per liter; after pasteurization, the extreme values were 0.21 and 0.34, and averaged 0.30 mg. per liter. The loss of thiamine during pasteurization was 9.1%.

C.F.H.

102. The Application of Chromatography to the Study of the Carotenoids of Human and Cow's Milk. S. Y. THOMPSON, S. K. KON, AND

E. H. MAWSON, Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Abstracts of papers read at the Proc. of the Biochem. Soc., Nov. 7, 1942, London. Biochem. Jour., 36, Nos. 10, 11 & 12 (combined): xvii. 1942.

The authors "have chromatographed numerous samples of human milk fat and have found that β -carotene contributes from 20 to 25% or even less of the total absorption at 451 m μ . In the majority of samples examined a pink band lies above that of β -carotene, chromatographically homogeneous with and showing the same absorption curve as crystalline lycopene, obtained from tomatoes.

"Shorthorn butter fat showed no lycopene zone. . . . In Guernsey fat there were three absorption zones between those of β -carotene and xanthophylls, one of which was pink and occupied the lycopene position. When 80 pounds of fresh tomatoes were given to a Guernsey cow during 6 days, the intensity of these zones rose from 4 to 16% of the total absorption. The pigment was identified as lycopene. In Guernsey milk β -carotene forms a smaller proportion of the total pigments than in Shorthorn milk."

A.O.C.

HERD MANAGEMENT

103. Dairy Management Problems. EARLE L. MOFFITT, Penn. State College. Milk Dealer, 32, No. 11: 32, 66-67. Aug., 1943.

Stating "that 95% of the success in operating a farm comes from the thought or headwork put into the business," the author stresses the factors essential for financial success. The need of accurate farm records, the use of the data such records reveal, and the careful account of all details mark the difference between success and failure. Farm management studies made by the Pennsylvania State College are cited that show that unless yearly farm sales equal at least 25% of the total farm investment, there is practically no chance to show a yearly profit. Values of \$15 to \$18 per acre of crops on a general farm and \$25 to \$35 per acre of crops on special farms are given as goals to attain if a profit is to be assured. Details of dairy farm management are itemized and the proper balance to maintain in accord with proven practices are cited. The need of proper allocation of time and effort to each phase of farm management is pointed out.

C.S.T.

ICE CREAM

104. Dried Whole Egg Powder. VI. Effect of Storage Temperature and Gas Packing on Keeping Quality. W. HAROLD WHITE, M. W. THISTLE, AND MARGARET REID, Natl. Res. Labs., Ottawa. Canad. Jour. Res., D, 21, No. 9: 271. 1943.

Dried whole egg powders were obtained from three different manufacturers and stored at temperatures of 45°, 60°, 75° and 90° F. for periods up

to six months. Quality was assessed by determination of fluorescence and potassium chloride values. At 75° F. the rate of deterioration was comparatively rapid and at 90° F. it was markedly so. To maintain quality during storage dried egg should be stored at a temperature of 60° F. or lower.

The effect on keeping quality of packing in nitrogen, carbon dioxide, under vacuum, or in the form of compressed tablets was studied. Carbon dioxide alone had beneficial effect. O.R.I.

105. **Dried Whole Egg Powder. VII. Effect of Temperature and Moisture on the Bacterial Content of Liquid and Dried Egg.** N. E. GIBBONS AND C. O. FULTON, Natl. Res. Labs., Ottawa. *Canad. Jour. Res., D*, 21, No. 10: 332. 1943.

The bacterial content of liquid egg increased rapidly after about 6 hours at 68° F., 12 hours at 60° F., 25 hours at 52° F. and two or three days at 45° F. At 38° F. there was little change for five or six days, followed by a very gradual increase.

The bacterial content of the dried egg powder was influenced by the number of bacteria in the melange, the drying temperature, the rate of cooling, the storage temperature and the moisture content. Low drying temperature and rapid cooling of the powder favored survival. On storage the bacterial mortality increased with increasing time and temperature. Up to 8.6%, moisture content had little effect on bacterial survival. At moisture levels above 5% there was an increase in the number of molds, particularly at 75° and 90° F. O.R.I.

106. **Acid Standardization.** A. D. BURKE, Ala. Polytechnic Inst., Auburn, Ala. *Ice Cream Field*, 43, No. 3: 64. 1943.

Early in the history of the ice cream industry it was considered good practice to "ripen" ice cream mixes and that a "livery" mix was believed to be the secret of good whipping, high yield and smooth-bodied ice cream. Experimenters early proved the fallacy of this concept and further showed that aging had little advantage beyond 24 hours.

The practice of adding certain salts to ice cream mixes as a basis of controlling mix viscosity, the author considers was the start of acid standardization. Five objections to "neutralization" are given but the author concludes that there are conditions which justify acid standardization or "neutralization."

Advantages listed for acid standardization are as follows: (1) provides uniform control of viscosity, (2) aids in whipping properties of high acid mixes, (3) improves melt down qualities of high acid mixes, (4) it helps intensify the apparent sweetness and (5) it may be of some benefit in retarding sandiness.

The author warns against over neutralization but considers it safe to reduce the acidity of ice cream mixes to about 0.18%. He suggests the following basis of calculating the correct value for acid standardization:

$$\frac{\text{Serum solids of mix}}{\text{Serum solids of milk}} \times \text{acidity of milk} = \begin{cases} \text{desired} \\ \text{standardized} \\ \text{acidity} \end{cases}$$

Directions are given for "neutralization" dependent upon weight of mix, its original acidity and the neutralizer used. W.C.C.

107. Bugaboo of Barriers. HAROLD F. PIERCE, London, Ontario. *Ice Cream Field*, 43, No. 3: 42. 1943.

Many of the practices necessitated by the war have been beneficial to the ice cream industry of Canada. Costs have been reduced because of territory exchanges, reduced dealer service and cabinet repairs as well as marked reduction in advertising it is claimed. Hope is expressed that certain of the advantageous practices will continue after the war.

"Bootlegging" has been a minor problem. Strict adherence to quotas has been practiced. Cereal substitutes for dried milk have been used and sugar substitutes such as honey and corn syrup are officially tabooed. Glucose, although still allowed, is practically unavailable.

Regret is expressed that advertising has been so drastically reduced. The author concludes that except for the question of good-will building, dealer supervision, advertising and dealer help the Canadian picture is satisfactory. W.C.C.

108. What Other Solids to Use? The Serum Solids Situation. C. C. FLORA, Va. Polytechnic Inst., Blacksburg, Va. *Ice Cream Field*, 43, No. 3: 19. 1943.

The author briefly discusses some of the problems resulting from wartime restrictions in the ice cream industry. He reviews the findings of various investigators and commercial operators as to what products can be used to replace serum solids in ice cream. He mentions a few of his findings which in general confirm those previously reported.

It is concluded that corn sugars, apple juices and syrups "offer possibilities for replacing part of the serum solids in ice cream." Oat flour (Avenex), Cincrose, soybean flour and wheat flour are also placed in this same category. W.C.C.

109. Serum Solids Substitutes. C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State Col., State College, Pa. *Ice Cream Field*, 43, No. 3: 14. 1943.

Experimental mixes which contained 10.5% fat, 8.4% serum solids, 15% sugar 60% of which was Frodex and 0.4% gelatin were prepared with vari-

ous flours by pasteurizing them at 160° F. for 20 minutes, since this was found to give better results than heating at 150° F. for 30 minutes. The results obtained confirmed the findings of others and further showed that Avenex or oat flour when used to the extent of 1% "gave as good or better results than 2% of the others."

They conclude that when over 1% oat flour is used a cereal flavor results but 2% soya flour also imparts a cereal flavor, whereas 2% of wheat, cake, or corn flour "caused no particular flavor defect in vanilla ice cream." Even "3 or 4% of ordinary wheat flour gave little indication of cereal taste."

The authors report that nothing except egg yolk and monoglyceride was found to improve overrun.

It is recommended that mixes containing cereal products be well agitated when used to insure the proper dispersion of part of the cereal which settles during standing.

W.C.C.

MILK

110. **Calculating Total Solids in Milk.** CHARLES W. LIVAK. *Natl. Butter and Cheese Jour.*, 35, No. 1: 13. Jan., 1944.

Samples of milk were taken in November, December and January and analyzed for fat and solids by the Mojonnier method. Specific gravity measurements were made with lactometers and a Westphal balance. Percentages of total solids were calculated by Sharp and Hart's equation. The total solids so calculated were 0.228 and 0.307% higher than the Mojonnier method indicated, depending upon whether Rueda's or a "theoretical" correction factor was used to convert specific gravity values to 86° F. with reference to water at 86° F.

W.V.P.

111. **Why Certified Milk Should be Retained.** J. HOWARD BROWN, Ph.D., Sc.D., Chairman, Council of the Amer. Assoc. of Med. Milk Commrs. *Milk Dealer*, 32, No. 10: 48-49. July, 1943.

The possibility of the elimination of certified milk by allowing only two grades of milk as a war measure is discussed and a plea is made for retention of "certified milk" as a medicinal food rather than a premium milk. Certified milk is controlled by the American Association of Medical Milk Commissions, Inc., a non-profit organization. This milk is not produced and marketed for financial profit and its methods and standards of production are rigorously controlled. Nutritional, chemical and sanitary differences are maintained as are special qualifications of herds licensed to produce such milk. Ninety per cent of the sales of certified milk are made upon the prescription or recommendations of qualified physicians who consider it as a product "which fills a need and exerts an influence far beyond what its

volume of production and consumption might seem to justify." Its continuation as such a distinct product is urged. C.S.T.

112. **Plant Cooling System for Milk Dealers.** ANONYMOUS. Milk Dealer, 32, No. 10: 32-33. July, 1943.

The adaptation of the cooling of water for refrigeration purposes to apply to flat-top dairy plants by use of a spray canopy system is recommended as a means of controlling and reducing plant building temperatures and as an auxiliary air-conditioning aid. The system advocated is by means of feed water pipes installed above the roof rather than under it, so that pipes require no insulation and can be operated with waste water from condenser coils at a nozzle pressure of 7 pounds. A spray pond covering the whole roof to protect it from solar heat, with a circulating system providing for recirculation, properly installed will reduce afternoon temperatures within the plant of a one-story flat-roofed building from 10 to 15 degrees. Such a system will also increase the life of the roof by stopping excessive expansion and contraction of roof material and loss of volatile oils from asphalt by hot sun. C.S.T.

113. **Making Quality Easy for Milk Producers.** PAUL H. MONDT. Milk Dealer, 32, No. 11: 30-31. Aug., 1943.

Farm labor as well as plant labor is critical. Therefore the producer needs all the help the plant operator can extend to promote continued production of quality milk. The producer should be educated as to the need of quality to prevent waste, promote edibility and use of dairy products, and to provide extra food. This can be done by means of posters, loaning such items as hair clippers, and by distributing whitewashing formulas and directions to farmers. Directions for the proper care of milking machines; blueprints on correct milk house construction, together with cement or building instructions; providing building forms for cooling tanks; aid in the purchase of supplies, cans, brushes, powders, etc.; and suggestions for the correct care of the cows, equipment and milk are discussed. C.S.T.

114. **Consumer's Problems of Every-Other-Day Milk Delivery.** GEORGE F. DOW. Milk Dealer, 32, No. 12: 33-34, 66-68. Sept., 1943.

The "other side," namely, the consumer's rather than the distributor's problems of every-other-day milk delivery is presented. Such factors as the keeping quality of milk, home refrigeration facilities and changes in source of supply are analyzed from a survey of 966 families in Portland and Westbrook, Maine. Consumer problems were souring of milk in hot weather, off-flavor development from standing outside or uncapped in home refrigerators, thickened cream layer, poorer keeping quality of raw milk as com-

pared with pasteurized, and the relation of time of delivery to the keeping quality of the milk delivered. The type of home storage of the milk was a factor in that ice refrigeration as compared with mechanical presented more of a quality and keeping problem. Detailed percentages of various complaints are given. On the whole the net change in milk purchased and consumed was negligible; quality was unaffected if adequate refrigeration was available in homes and if the time of delivery was adjusted. Seventy-six per cent received their milk from distributors with established home deliveries and but few changed dealers, thus demonstrating that consumers were willing to cooperate with the plan to conserve trucks and labor as a war measure.

C.S.T.

115. Pioneer Milk Pasteurizing Plant. FRED W. MEEN. Milk Dealer, 33, No. 2: 39-40. Nov., 1943.

The description of equipment, methods used and of delivery and sales methods followed in the organization and starting of what is said to be the first commercial bottled fluid milk pasteurization plant established in the United States at Rush City, Minnesota, in 1897, is of historical interest to the milk industry.

C.S.T.

116. Securing Customer Cooperation to Ease Milk Delivery Problem. C. W. ESMOND, G. P. Gundlach & Co., Cincinnati, Ohio. Milk Dealer, 33, No. 2: 48-50. Nov., 1943.

With the necessarily curtailed service to all milk customers in effect as a wartime emergency measure, the need for customer cooperation—always essential for best dealer-customer relationship—is now more than ever a “must” if confusion, complaints and loss of customers are to be avoided. An intelligent, systematic and continuous educational program to acquaint the customer with the problems confronting the dealer together with a recognition of the consumer’s unaccustomed inconveniences and problems will go far towards solving present-day wartime milk delivery problems, as well as to meet post-war competition and “to retain and to improve upon all the economical distribution methods of war time, such as the elimination of special delivery, standing orders, conservation of bottles, reduced number of deliveries per week, minimizing the overlapping of routes, collecting at lowest possible cost, saving unnecessary steps for milkmen, etc.,” and thus retain and maintain the closer and more friendly dealer-customer cooperation developed as a wartime necessity.

C.S.T.

117. Improving Chocolate Drinks. B. E. HORRALL, Purdue Agr. Expt. Sta., Lafayette, Ind., AND M. O. MAUGHAN, Amer. Dry Milk Inst., Chicago, Ill. Milk Dealer, 33, No. 3: 24-25, 48. Dec., 1943.

A survey cited by the authors revealed that 80% of all chocolate milk sales was a “plus” not a “replacement” volume, indicating that chocolate

increases the palatability of milk to many consumers. Studies therefore were undertaken "to (a) improve the flavor and (b) increase the nutritive value of chocolate drinks." Experimental technique was to use various percentages of milk fat where milk solids-not-fat were added to different lots of whole and separated milks plus four different chocolate products and sugar. Cream, butter and butteroil were used for desired fat combinations and spray-dried skim milk powder for solids-not-fat additions. Six experiments were conducted with varying percentages of milk fat to which added milk solids-not-fat were combined. All samples were tested by seven or more judges. Added milk solids-not-fat to the extent of 3% improved all commercial chocolate milk drinks. A 2% butterfat product enriched with 3% milk solids-not-fat was adjudged the best-flavored product, followed closely by the 3% and 1% butterfat products to which 3% milk solids-not-fat were added. A commercial formula for addition of spray-dried skim milk powder to either a skim milk or reconstituted milk mixture is given.

C.S.T.

118. The Function of the Laboratory in the Control of Milk Supplies.

F. W. FABIAN, Res. Prof. Bact., Mich. State Col., East Lansing, Mich. Jour. Milk Technol., 6, No. 5: 278. Sept.-Oct., 1943.

A good discussion is given of the various laboratory tests used in the dairy industry and their relative values in determining the sanitary quality of milk.

The author concludes that "it looks as though we are going to use the plate method less since it is expensive, time-consuming and tells only a part of the story. In its stead we shall substitute the direct microscopic test supported by other tests. For raw milk, we shall use the odor test, the sediment test, and either the direct microscopic or the resazurin test or both. For pasteurized milk, we shall use the direct microscopic method supplemented by the coliform and phosphatase tests."

The author believes it will be some time yet before laboratory tests will be developed to the stage where they will supplant the milk inspector. He does think, however, that the future inspector will be better trained and will be a combination inspector and laboratory man, doing most of his own laboratory work.

The ideal situation is to use the milk inspector to locate the visible dirt and the laboratory to find the invisible dirt. "They should supplement rather than supplant each other."

L.H.B.

119. Care of Milking Machines. C. K. JOHNS, Central Expt. Farm, Ottawa, Canada. Jour. Milk Technol., 6, No. 5: 274. Sept.-Oct., 1943.

In studies made at the Central Experiment Farm, it was found possible to keep milking machine rubber parts in good sanitary condition without

the use of hot water after each milking. This method was developed in 1930 and has been in use ever since.

During a period of nearly twelve years, more than 1200 samples of the mixed raw milk (nights and mornings) were taken from the pasteurizing vat prior to pasteurization. More than 70% of the agar plate counts were below 10,000 per ml. and more than 95% were below 50,000 per ml.

The method used is very simple and is as follows:

Immediately after milking at least two gallons of clean, cold or luke-warm water are drawn through each unit, raising and lowering the teat cups several times to obtain an air-brush effect. The outer surfaces of the teat cup assembly are then cleaned off with a brush and hot detergent, hung on a solution rack and filled with a weak (0.5%) lye solution until the next milking. At that time the solution is drained out and the units re-assembled and ready for use. It has not been found necessary to rinse the units to remove traces of lye solution, but a chlorine rinse at this time could be drawn through the unit to advantage. At intervals of one or two weeks, the rubber parts are dismantled, primarily to prevent adherence to the metal, and for thorough inspection of the tubing and liners to note their condition. Worn out tubing and liners are replaced at this time, and if necessary, liners are trimmed to proper length. The tubes and liners are brushed in hot detergent solution and rinsed before re-assembling.

The lye solution has an advantage over the chlorine soak solution in that the lye saponifies any butterfat and dissolves any casein which might be present, thus leaving the rubber parts physically clean. The lye solution is also a good germicide and prevents the growth of bacteria in the tubes.

L.H.B.

MISCELLANEOUS

120. Proper Paint Helps Solve Surface Maintenance Problems. ANONYMOUS. Milk Dealer, 32, No. 11: 29-58. Aug., 1943.

Stressing the need of proper maintenance of all milk plant equipment, the paint-up program, with paint still available, can and should be regularly maintained, particularly since the milk plant must of necessity operate under conditions of moisture and steam condensation especially destructive to ordinary paint.

The need of special moisture and acid-resistant protection from corrosion and fungus and mold growths by use of paints possessing such properties is stressed. The use of proper white and light-tinted paints for increased illumination which reduces eye strain and fatigue, aids employee morale and decreases accidents. In some plants accidents have been eliminated 100% by selection of a high light-reflecting paint. A table of reflection values for different colors ranging from 11 to 25% for dark green to 80-89%

for white is given. The suggestion is made that wherever practical, increased light reflection is obtained by painting floors white or light colored.
C.S.T.

121. **First Aid for Ailing Cold Storage Rooms.** ANONYMOUS. Milk Dealer, 32, No. 11: 26. Aug., 1943.

Citing a recent survey made by the Refrigeration Service Engineers Society that approximately half of the refrigeration service men have left their former jobs, the author stresses the need for all fluid milk plants to keep their refrigerating machinery and cold storage rooms at top efficiency lest spoiled products through breakdowns with resulting higher operating costs result. A new type of circulating fan which requires no skilled mechanic to install provides for better air circulation on a new principle of blowing the cold air from the refrigerated coils up to the ceiling, down the walls and up the center, moving all the air in the room to maintain an even and equalized temperature. Such a movement of air promotes dryness, dispels odors quickly, prevents frosting of coils, retards mold growth and reduces cost. A diagram of air currents after installation of fan graphically illustrates diffusion of air.
C.S.T.

122. **National Control or Free Competition for Britain's Milk Industry.** GEORGE WALWORTH. Milk Dealer, 33, No. 2: 64-66. Nov., 1943.

Whether government control of Britain's milk industry after the war is made permanent or whether the industry is permitted to revert to pre-war free competition is contingent upon the extent to which the present essential processing and price-control measures are voluntarily retained by private enterprise. To insure national nutritional requirements, proper price relationship as to milk quality and its utilization, the maintenance of proper balance between liquid milk and processed products consumption, economical maintenance of sales and distribution and milk quality control are factors the British milk industry must face and solve satisfactorily to avoid national post-war control of milk. The establishment of a milk control council by the industry with power to regulate such factors in the interest of consumers, as well as to insure adequate returns to the industry, is advocated. This British question is of importance to the United States in that post-war international relationships are involved and concern all milk-producing nations. "Liquid milk occupies the key position in Britain's agriculture but dairy produce will be of considerable significance in overseas trading with Britain."
C.S.T.

123. **Use of Chlorine on the Farm.** JACK KEENAN, Gen. Lab. Div., Penna. Salt Mfg. Co., Philadelphia, Pa. Milk Dealer, 33, No. 2: 52-57. Nov., 1943.

Since milk quality control starts at the farm, the utilization of chlorine

for cleaning all utensils, for washing cows and milkers' hands, rinsing cans, etc., is of vital importance. Methods of preparing standard chlorine solutions and rinses are given for all such uses. Stress is laid upon need of using chlorine in washing cows' udders and teats to prevent infection, and in cleaning milking machines to prevent spread of disease. The author concludes that "next to sunshine, chlorine may be said to be our best bactericide" and that its use is safe, inexpensive and particularly adapted to use on the farm. C.S.T.

124. Plastics. ANONYMOUS. *Milk Dealer*, 33, No. 3: 26-27. Dec., 1943.

With the advent of war, substitute materials for use in dairy plant equipment became necessary. Since then substitutes for substitutes have appeared. The use of plastics, since they are light, unbreakable, clear and impart no flavor or odor, in making both old and new milk plant equipment, would seem to afford highly interesting possibilities. Particularly interesting is the possibility of supplanting standard tubing and piping with plastic material. C.S.T.

125. Tin in the Dairy Industry. JULIA DEXTER, Battelle Memorial Inst., Columbus, Ohio. *Milk Dealer*, 33, No. 3: 34, 46-48. Dec., 1943.

The use of tin in the dairy industry from the tin milk pail of the farmer to the tin can on the grocery store shelf through all the intermediate steps of collection, transportation, cooling, storing and packaging in tin-coated equipment is described and its present critical status discussed. New methods of utilizing tin as a coating which will favor retention of tin in postwar use in the dairy industry are described. Electrolytic plating of tin by different new methods; the use of white bronze plating to tin copper or in combination with nickel, tin spraying and new and better tin foil for wrapping dairy products are postwar possibilities which "point the way to better and far heavier tin coatings than have been possible in the past. Repairs and relining are facilitated in many cases by electrolytic tinning means. Tin-coated steel is the most inexpensive material of construction for dairy equipment." C.S.T.

126. Steel for Insulation. JAMES G. MACORMACK, Amer. Flange & Mfg. Co., Inc., and C. T. Hogan & Co., Inc., New York. *Ice Cream Field*, 43, No. 3: 34. 1943.

The author claims that Ferro-Therm steel insulation offers increased efficiency and economy to the ice cream industry. Ferro-Therm steel insulation sheets are made of thin gauge commercial steel with a hot dipped lead and tin alloy coating "which reflects 90 to 95% of radiant heat. These sheets are installed with approximately one-half inch air space on either

side, broken up by separators every 24 to 32 inches. Ferro-Therm steel insulation was first introduced in 1933-34 but it has been extensively used since 1937 in many industries requiring refrigeration.

Eight sheets of Ferro-Therm insulation in an overall thickness of 4 inches is equivalent to 8 inches of corkboard. Ferro-Therm sheets from 26 U.S.S. gauge to 18 U.S.S. gauge are used for the outside linings for rigidity, while all intermediate steel sheets are No. 38 U.S.S. gauge and weigh 0.25 pound per square foot.

Fifteen sheets within a thickness of 6 inches used on a "dry ice" storage cabinet resulted in a loss of 1.8% for 24 hours whereas 8 inches of corkboard insulation on the same type of cabinet resulted in 3% loss per 24 hours. The Ferro-Therm container weighed 1100 pounds and held 2938 pounds of dry ice, whereas the corkboard container weighed 1500 pounds and held 2250 pounds of dry ice.

The degree of luster or optical brightness has little to do with the ability of the surface to reflect heat or infra red rays. Tests by the U. S. Bureau of Standards show that reflective insulating sheets exposed to industrial atmosphere for two years until they became a dull gray-black had 97% of the reflectivity of radiant heat they had when new and bright. Moisture condensation is very slight in a Ferro-Therm construction. The heat storage capacity of the steel insulation is relatively low, which permits more rapid temperature pull down than with the usual type of mass insulation.

W.C.C.

ABSTRACTS OF LITERATURE

BACTERIOLOGY

127. Further Studies on Coliform Bacteria Serologically Related to the Genus *Salmonella*. P. R. EDWARDS, W. B. CHERRY, AND D. W. BRUNER, Ky. Agr. Expt. Sta., Lexington. Jour. Infect. Dis., 73, No. 3: 229-238. Nov.-Dec., 1943.

In a continuation of work reported earlier (Jour. Dairy Sci., 25, No. 6: A144. June, 1942) the serological properties of 44 coliform cultures were studied. All of the cultures studied were considered pathogenic. An antigenic analysis revealed that the 44 cultures were divisible into 14 serological types and these types were divisible into 11 somatic groups. With the exception of 3 cultures, the H antigens of all of the types resembled those of *Salmonella düsseldorf* and *S. cerro*. The H antigens of the other 3 cultures resembled those of *S. enteritidis*. Antigenic formulas, based in part on the Kaufmann-White schema for the salmonellas, are given for the 14 types.

J.F.C.

CHEESE

128. Consistency of Cheese Curd at Pitching and Grinding. F. M. V. COPPEN. Dairy Indus., 8, No. 9: 488. Sept., 1943.

The author discusses a number of control tests which are employed at different stages in Cheddar cheese manufacture. Data are given and the relative merits of these tests presented.

D.V.J.

129. Oil Separation in Processed Cheese. J. L. PALMER and W. H. SLY. Dairy Indus., 8, No. 8: 427. Aug., 1943.

In the manufacture of processed cheese much can be done to avoid oil separation during storage and distribution. It is pointed out that the ripeness of the cheese used for processing is a very important factor. Very ripe cheese frequently results in loose, grainy and oiled-off processed cheese while very fresh cheese produces a stable emulsion.

The stabilizing salt (called melting salt in England) is also a very important factor and according to these authors sodium citrate alone or a suitable blend of sodium citrate and phosphates produce the best results. Sodium metaphosphate is of questionable value. When the pH of a processed cheese is over 5.8 to 6.0 or below 5.4, there is more danger of oil separation. It is also pointed out that prolonged or violent agitation resulting in aeration will have a tendency to break down the emulsion. A continuous process is more desirable than a batch system.

D.V.J.

DISEASE

130. **Studies on Epidemic Diarrhea of the New-Born: Isolation of a Filtrable Agent Causing Diarrhea in Calves.** JACOB S. LIGHT, M.D., AND HORACE L. HODES, Lt. MC, USNR, Johns Hopkins Univ. and Sydenham Hosp., Baltimore City Health Dept., Baltimore, Md. *Amer. Jour. Pub. Health*, 33, No. 12: 1451-1454. 1943.

In connection with four separate epidemics of diarrhea of the new-born a filtrable agent has been isolated which regularly produces diarrhea in calves. In the attempts so far made, this agent has not been isolated from the stools of normal infants or normal calves. The evidence suggests, though it is not conclusive, that the agent may be a cause of epidemic diarrhea of the new-born.

M.W.Y.

131. **Use of Modern Laboratory Aids in the Investigation of a Typhoid Fever Outbreak.** EDWARD R. SCHLESINGER, Asst. Dist. Health Officer, N. Y. State Dept. Health, Albany, N. Y. *Amer. Jour. Pub. Health*, 33, No. 10: 1257-1262. 1943.

An outbreak of 27 cases of typhoid fever is described which occurred in Oswego County, New York, in 1941 and 1942. Twenty-three of the cases were probably due to the ingestion of curd or fresh cheese at a local factory or were secondary to such cases. The cases occurred over a period of 17 months. Bacteriophage typing separated the type C cases, due to the contaminated curd and cheese from type A cases traced to food prepared by two type A typhoid carriers. Two typhoid carriers were found among the dairy farmers supplying the cheese factory. Typing showed one to be type C and eliminated the other as the possible source of the outbreak, despite the fact that epidemiological evidence alone pointed more forcefully to the latter as the carrier responsible for the outbreak.

M.W.Y.

132. **What Is Known on Undulant Fever.** K. G. WECKEL, Assoc. Prof. of Dairy Indus., Univ. Wis. *Milk Plant Monthly*, 32, No. 7: 41-42. 1943.

The disease "undulant" fever, deriving its name from its characteristic of recurring at intervals or in cycles, is known also as Brucellosis, intermittent typhoid, Malta or goat fever or subcontinuous fever depending in part on locality and observations. The disease has its origin in animals. The bacteria responsible for it among cattle is known as *Brucella abortus*; among sheep and goats as *Brucella melitensis*; and among swine as *Brucella suis*. Any type may cause undulant fever in man but that from *Brucella melitensis*, which has seldom been diagnosed in man in the United States, is the most fatal. *Br. abortus* are discharged through uterine secretions or, when the udder tissue is infected, through the milk. Humans consuming

raw milk may contract the disease but relatively this seldom occurs. The eradication program has reduced the incidence of the disease to a low of probably 2% or less. Three general methods are used in eradication: (1) Test and slaughter, (2) Test and segregation, and (3) Calfhood vaccination.

The incidence of undulant fever in humans is greatest among handlers of livestock and slaughterhouse or meat packing plant employees. The disease among humans is not contagious. There have been but very few recorded outbreaks of Brucellosis traced to milk and those to raw milk. No outbreaks have been reported from consuming pasteurized milk. Hence, the program of eradication and pasteurization minimizes the economic loss caused by the disease on farms and assures the consumer of a safe milk supply.

G.M.T.

FEEDS AND FEEDING

133. **Physiological Requirements and Utilization of Protein and Energy by Growing Dairy Cattle.** E. G. RITZMAN AND N. F. COLOVOS. N. H. Agr. Expt. Sta. Tech. Bul. 80. 59 pages. Aug., 1943.

An intensive study on the energy and protein requirements for growth of dairy cattle, from birth to approximate maturity. Eleven pure bred Holstein heifers were used in the following age groupings: Birth to 4 months, 3 heifers; 4-36 months, 4 heifers; 8-27 months, 4 heifers.

This bulletin lacks an adequate summary and it is difficult at times for the reader to distinguish between conclusions drawn from original data, and statements based on other work having a bearing on the results. The following statements, for the most part direct quotations, have been selected as being of particular significance.

A very close agreement was found between individuals in their basal metabolism at approximately the same age. A high pitch of tissue activity prevailed for about a week after birth; this was followed by a sudden drop, after which further decline was quite steady and gradual. This declining rate in tissue activity was reflected by a periodical decline in growth rate as indicated by daily gains in live weight in relation to total weight of the individual.

Certain of the results demonstrated the critical importance of the time factor in growth. Aging is almost purely a question of time, and maturity is reached in the span allotted for the species whether food is scarce or abundant.

An offshoot from the main investigation was a study of the effect of accessory illumination on metabolism. The conclusion that seemed most probable was that any effect of visual light on metabolism is immediate and prevails only as long as the (nerve) exciting effect of light is present.

The biological value of protein depends not only on the character of its nitrogenous constituents, or on the level of the protein intake, but also on

the physiological adaptability of the individual to utilize it. . . . The true measure of value of a feed for a given purpose must be determined by the individual which uses it most efficiently. The results do not suggest any beneficial results from the so-called associative effects of protein from different sources, but rather a low order of biological value of cereal proteins for growth.

Protein utilization values secured indicate that the protein of hay had a surprisingly high value for growth (practically the same as grain); that corn had a low value, that linseed meal was somewhat better than cottonseed meal, and that barley was about equal to oats.

Efficiency of protein utilization was determined as much by the inherent growth rate potential of the individual as by the available supply of food protein.

Relatively great differences may exist in protein utilization between individuals of similar age and type or breed, and maintained under like conditions.

The comparative uniformity of the decline in protein conversion indicates that it is impossible to force growth beyond these declining inherited limits.

The influence of hereditary limitations to storage of protein for growth appears to be the deciding factor as to the form in which available food energy in excess above maintenance is stored by the body.

Quantitative statements of requirements of protein and energy appear in detail in a series of tables which do not lend themselves to a simple summary statement. The reader is therefore referred to the original article for further specific information.

17 tables, 43 literature references.

J.G.A.

134. Meeting the Protein Requirements of Dairy Cows. H. B. ELLENBERGER. Vt. Agr. Expt. Sta. Pamphlet No. 1. 12 pages. March, 1943.

A non-technical discussion of the subject in the light of the present protein shortage. The need for good pasture supplemented by a simple grain mixture such as corn and oats, is stressed. The writer's opinion is that during the present emergency a concentrate mixture containing not more than 16% protein, if liberally fed, should serve most dairy needs, including winter feeding. The importance of storing ample supplies of roughage of the highest possible quality is strongly emphasized.

J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

135. Milk in the National Food Program. W. E. KRAUSS, Chief, Dairy Dept., Ohio Agr. Expt. Sta., Wooster, O. Milk Plant Monthly, 32, No. 9: 34. 1943.

Emergency food problems involve an adequate supply of calories, pro-

tein, minerals and vitamins from sources people have been accustomed to using and in keeping with the agricultural system which makes efficient use of land labor and feed. In 1942, about 40% of the food energy, 57% of the protein and 73% of the fat were from livestock products. Three-fourths of the calcium and about one-half of the riboflavin in the civilian food supply were from milk products. Considering the output of calories and protein from concentrates fed, the utilization of labor for the production of protein, the protein obtained per acre, the minerals and vitamins, milk ranks at the top followed closely by eggs. Milk and egg production are, therefore, emphasized. Indications are that the demand for milk may be maintained or even increased.

G.M.T.

136. **Chemical and Biological Stability of Crystalline Vitamins D₂ and D₃ and Their Derivatives.** W. HUBER AND O. W. BARLOW, Res. Labs., Winthrop Chem. Co., Inc., Rensselaer, New York. *Jour. Biol. Chem.*, 149, No. 1: 125. July, 1943.

All forms of Vitamin D, including Vitamin D₂ (irradiated ergosterol) in a crystalline form, or when absorbed on casein (ertron), Vitamin D₃ (activated 7-dehydrocholesterol), as well as Vitamin D from natural sources, are susceptible to destruction through oxidation as shown by changes in their melting points and specific rotations. This decomposition is more rapid when they are stored in the dry form rather than as emulsions. The various nitrobenzoic acid esters of Vitamins D₂ and D₃ are more stable for they showed no apparent decomposition after being stored for five years.

When an emulsion of Vitamin D₂ in propylene glycol (drisdol) was diluted with water it lost as much as 75% of its potency during the period of bioassay, but when diluted with milk or propylene glycol it showed no deterioration.

Market milk fortified with Vitamin D₂ showed no apparent loss of potency when held for 8 days and evaporated milk, similarly fortified, showed no loss of Vitamin D after storage periods ranging from 6 to 15 months.

A.O.C.

137. **Some Medical Aspects of Protein Foods.** FREDRICK J. STARE AND GEORGE W. THORN, Schools of Pub. Health and Medicine, Harvard Univ.; Med. Serv., Peter Bent Brigham Hosp., Boston, Mass. *Amer. Jour. Pub. Health*, 33, No. 12: 1444-1450. 1943.

The protein requirement of man cannot in the present state of knowledge be quantitatively stated in terms of amino acids, under certain standardized conditions. Calorie intake from carbohydrate and fat spares protein, and in the presence of sufficient calories from non-protein sources, the amount of protein in the diet of an active adult may be safely reduced to 50 grams per day, of which as little as 5 grams may be in the form of animal protein.

The protein requirement is not increased in exercise, and physical fitness and efficiency are not impaired or improved on low protein diets adequate in other nutrients. Substituting protein for some of the carbohydrate in the common American breakfast is a feasible way to prevent mid-morning symptoms of fatigue. High protein diets are not harmful to the normal adult and are of definite therapeutic value in many diseases and particularly in convalescence. Certain less common protein foods exist which are of high nutritional value, and which could be used with considerable value in post-war feeding operations. Protein foods are excellent sources of nutrients other than proteins, and we cannot reduce the protein of a diet unless at the same time we provide the other essential factors contributed by protein foods.

M.W.Y.

MILK

138. **The Place of the Dairy Industry in Postwar America.** CHRIS L. CHRISTENSEN, Vice Pres., The Celotex Corp. *Milk Dealer*, 33, No. 4: 70-71. Jan., 1944.

Fluid milk production and distribution as a postwar product will experience many changes. Standardization of milk inspection laws and regulations; economies in transportation both from farm and to consumer and the possible inauguration of partial condensation by milk plants before delivery to consumers are postwar changes in the processing field. In the production field (1) improved feeding methods of both hay and grain by use of recommended feeding procedures to improve quality and nutrition of milk as well as quantity; (2) improved soil and soil cultivation methods; (3) use of commercial nitrogen fertilizers for increasing pasture and hay production; (4) herd improvement through use of artificial breeding; (5) improved dairy barns and housing facilities through better designing; and (6) general reduction of milking time and improved equipment will all be factors to consider as a postwar problem in the dairy industry. C.S.T.

139. **A Technical Survey of Commercial Cultured Skim Milk or Buttermilk Manufacture in the United States.** F. V. KOSIKOWSKY, Dept. of Dairy Indus., Col. of Agr., Cornell Univ., Ithaca, N. Y. *Milk Dealer*, 33, No. 4: 25-27, 44-46. Jan., 1944.

A cross-section survey of 45 dairy plants through the United States made by use of questionnaire was basis of this study. A range of from 3,650 to 12.5 million quarts with a total of 25.6 million quarts of cultured buttermilk annually was represented in the survey. Principal factors studied were (1) proportionate relationship of cultured buttermilk to whole milk sold ranging from 1.5 to 14.83% with average of 2.59%; (2) source of starter—88.9% commercial starter used; (3) amount of starter used ranging from

1.28 to 2.17% with average of 1.84%; (4) amount of added milk fat used ranging from 1.00 to 1.69% with average of 1.44%; (5) temperature and range of holding time for pasteurization, temperatures used ranging from 160 to 190° F. for 30 to 37 minutes; (6) temperature and time of incubation ranging from 65° to 75° F. and from 10 to 16 hours held; and (7) acidity of curd when broken, cooled, and stored, ranging from 0.67 to 0.85%. Other factors studied were addition of salt, gelatin, butter, and customer complaints. Wheying-off and viscosity were deemed greatest problems by the author.

C.S.T.

140. **Addition of Citric Acid to Improve the Flavor of Cultured Buttermilk.** J. A. NELSON, Agr. Expt. Sta., Montana State College. Milk Dealer, 33, No. 4: 32. Jan., 1944.

The addition of citric acid to cultures used in producing cultured buttermilk, similar to use of citric acid in butter cultures, was studied in this experiment. Check sample of milk did not have citric acid added to cultures used but otherwise the samples were processed and treated similarly. The creatin test to determine quantitatively the acetylmethylcarbinol and diacetyl development showed in favor of citric acid cultures. Desirable flavor characteristics in all cultures to which citric acid was added were declared present by two competent culture judges. The author concludes "that the flavor of cultured buttermilk could be improved by the addition of citric acid to the culture milk before inoculation."

C.S.T.

141. **Agreeable Manner Will Maintain Doorstep Delivery.** C. W. ES-MOND. Milk Plant Monthly, 32, No. 8: 29-30. 1943.

The author points out the importance of keeping the milk customer satisfied. Disgruntled milk customers do not really object to curtailment of deliveries; to the prompt return of milk bottles; to the prompt payment of milk bills; to the delivery of uniform quantities of milk daily so as to eliminate returns, or to other reasonable practices approved by good dairy management, but do object to the brusque, abrupt and blunt methods used by some milk men in putting these changes into effect. Since the customer is the ultimate boss not only of the milk men themselves, but of the dairy industry, it behooves the management to insist upon the milk men handling customers with "kid gloves." The customer will continue to prefer doorstep delivery if it is done at reasonable cost, and in an agreeable manner. Agreeable manner in doorstep delivery will not only insure the job of the milk man, but in the end will help to insure the job of all other milk men as well.

G.M.T.

142. **Dye Reduction Tests for Heat Treated Milk.** A. L. PROVEN AND A. ROWLANDS. Dairy Indus., 8, No. 12: 693. Dec., 1943.

The authors do not recommend the colony count or the coliform test as

a means of controlling the keeping quality of heat treated milk. They suggest a half-hour methylene blue or resazurin test at 37° C. applied to samples which have been stored at 18° C. from 3 P.M. on the day of distribution until 10 A.M. the following day. This method can be relied upon to detect the majority of samples with an unsatisfactory keeping quality.

D.V.J.

143. **The Acidity Test Compared with the 10-Minute Resazurin Test and the Methylene Blue Test.** G. E. JONES AND H. BARKWORTH. Dairy Indus., 8, No. 11: 635. Nov., 1943.

A very significant correlation was found between the 10-minute resazurin test and the acidity of milk. The authors observed and presented data to support the fact that the 10-minute resazurin test is more closely affected by changes in acidity than is the methylene-blue test.

D.V.J.

144. **The Relative Keeping Qualities of Evening, Morning and Mixed Milk.** E. W. ERSKINE, B. M. FISCHER, S. M. L. SMITH, AND J. G. DAVIS. Dairy Indus., 8, No. 11: 618. Nov., 1943.

The authors found no marked differences in the bacteriological qualities of evening, morning and mixed milk. The methods used for this analysis were the "clot on boiling," methylene blue, resazurin, plate count and coli tests.

D.V.J.

145. **The Use of the Resazurin Comparator in Artificial Light.** J. G. DAVIS AND L. G. NEWLAND. Dairy Indus., 8, No. 10: 555. Oct., 1943.

In reading resazurin tests with the resazurin comparator daylight was found to be most suitable from all standpoints. However, low-pressure mercury vapor fluorescent tubular lamps gave very comparable results but were more fatiguing to the operator's eyes. In using this type of artificial light, it is desirable to exclude other extraneous light.

D.V.J.

146. **Thermoduric Organisms in Milk.** C. S. MORRIS AND M. EDWARDS. Dairy Indus., 8, No. 10: 550. Oct., 1943.

It is pointed out that heat-resistant types of organisms in milk can usually be traced back to a lack of sanitation on the farm or inefficient cleaning of milk cans at the creamery. Heat resistant coliform organisms may come from dried films on utensils or from contaminated water supplies.

Tests on raw milk are of little value in detecting thermoduric organisms. The author suggests that the pasteurized milk be examined by the following tests: 1. A phosphatase test immediately after pasteurization or when milk arrives at laboratory. Hold the milk for 24 hours at 18° C. and then run,

(a) a plate count at 30° C. or 37° C. on yeastrel milk agar (yeast extract milk agar), (b) a resazurin test at 37° C., and (c) a presumptive coliform test.

D.V.J.

147. **The Resazurin Test for Sterility of Milk Cans.** J. G. DAVIS AND D. W. WATSON. *Dairy Indus.*, 8, No. 8: 415. Aug., 1943.

The authors attempted several procedures of holding rinse solutions prior to testing them by the standard resazurin technique. Plate counts were used as a basis of comparison. The most satisfactory procedure of those tried was the following: One ml. of rinse is inoculated into 10 ml. of separated milk and incubated at 22° C. in a water bath or large tank in a heated room for 24 hours. At this time a standard resazurin test is made at 37° C. Any sample which reduces to a disc number of 5 or less in 30 minutes is regarded as unsatisfactory and probably has a count of more than 500 per ml. of rinse.

D.V.J.

148. **Cryophilic Bacteria in Relation to Milk Can Sterility Tests.** G. F. V. MORGAN. *Dairy Indus.*, 8, No. 8: 411. Aug., 1943.

The author found that the cryophilic organisms, isolated from milk cans after mechanical washing, were of a type usually associated with water. He points out that the detergent tank in the can washer frequently is the source of contamination and, therefore, should be cleaned after each day's operation. The rinse tanks on can washers are much less liable to become a problem. However, it is extremely important to keep the entire washer clean if contamination is to be avoided.

D.V.J.

149. **Rapid Platform Tests.** H. BARKWORTH, J. G. DAVIS, J. W. EDGELL, A. ROWLANDS, AND D. W. WATSON. *Dairy Indus.*, 8, No. 5: 215. May, 1943.

In an attempt to find suitable tests for detecting unsatisfactory milks at the receiving platform, the authors investigated the following methods: smell and taste, clot on boiling, alizarin-alcohol, titratable acidity, pH, resazurin (10 minutes and 1 hour) and methylene blue. Although most of these tests had certain desirable characteristics, the 10-minute resazurin test was found to be most suitable for the purpose.

D.V.J.

150. **Dye Reduction in Milk Related to Eh, pH and Dissolved Gases.** J. M. FRAYER. *Vt. Agr. Expt. Sta. Bul.* 498. Sept., 1942.

This publication contributes fundamental facts regarding the physiological processes involved in the application of the resazurin and the methylene blue tests for milk quality. The results as summarized are as follows:

"The reduction processes of the resazurin and methylene blue tests were

followed coordinately with Eh, pH and dissolved gas determinations. The results secured seem to warrant the following conclusions:

"1. There is little relationship between the moment of dye reduction and pH, oxygen depletion and pH changes not necessarily proceeding coincidentally.

"2. The bacterial growth phase at the moment of test initiation has much to do with the shape of the time: potential curve.

"3. The Eh level at the resazurin-pink stage of reduction is much more variable than it is when fully reduced.

"4. Most milks reduce resazurin to the pink stage at reduced oxygen levels, no case having as yet been observed of complete visual reduction of either dye in the presence of more than a minimum of oxygen.

"5. Milks held at 40° F. or less for 24 more hours tend to attain the resazurin-pink stage at higher Eh levels than when fresh.

"6. It can be safely assumed: (a) that all cell types found in milk have the same influence on dye reduction and gas depletion; (b) that oxygen is the only gas involved in the reaction; (c) that all unusual color modifications result from harmful abnormalities.

"7. The stage at which the carbon dioxide content is significantly increased is often accompanied by a rapid decline in both Eh and pH and by imminent color reduction.

"8. A low count milk sample to which resazurin is added, when exposed to bright sunlight and unaccompanied by metabolic activity previous to incubation, behaves as a rule as does a sample of poor milk in respect to the rapidity of oxygen depletion, of negative Eh swing and of color fading.

"Because of the relatively high sensitivity of the resazurin dye and the possible occurrence of color changes resulting from variations in factors other than those attributable to a high bacterial content and/or to physiological or pathological abnormalities, the opinion is advanced that, in the absence of microscopic confirmation, the methylene blue test for milk quality is likely to afford better results in the hands of the average milk plant operator than are any of the known modifications of the resazurin test."

P.H.T.

151. Mold Growth in Composite Milk Samples. J. M. FRAYER, Vt. Agr. Expt. Sta., Burlington, Vt. Pamphlet No. 2. April, 1943.

Mold growth in composite milk samples is objectionable because certain types of mold elaborate enzymes which may hydrolyze fat and consequently may lower the test as much as 0.3 to 0.4%. Mold growth also interferes with adequate sample mixing and pipetting.

To control this problem the mold must be prevented from getting into the sample. This can be done by using clean methods in handling the samples, by thoroughly cleansing all sample bottles, stoppers and storage boxes be-

tween each sampling period, and by adequate germicidal treatment of sample bottles and stoppers.

P.H.T.

152. **Bi-Monthly or Monthly Testing at Milk Plants.** D. W. WHITMAN, R. O. SLACK, AND E. O. HERREID, Vt. Agr. Expt. Sta., Burlington, Vt. Vt. Agr. Expt. Sta. Bul. 502. June, 1943.

As a labor-saving measure, composite samples may be tested monthly instead of bimonthly provided the bottles are kept tightly stoppered and are stored at 50° F. or below while not in use. It is also important to keep the samples free from mold growth and to properly prepare the samples for testing. Tests conducted in a commercial milk plant supplied by 113 patrons showed that the bi-monthly composites yielded only slightly more fat than the monthly composites. The preserved composite samples were prepared for testing by warming to 95–100° F. in a water bath at 107–110° F. Any adherent cream was carefully brushed loose. The samples were mixed by pouring four times. After pipetting, the warmed milk was permitted to cool to 70° F. before 15 ml. of sulfuric acid at 70° F. was added to the sample. Mixing of the acid and milk was done in a mechanical shaking device. The tests were read to 0.025% but were recorded to the nearest 0.1%.

P.H.T.

PHYSIOLOGY

153. **The Effects of Mild Hyperthyroidism on Growing Animals of Four Species.** M. KOGER AND C. W. TURNER. Mo. Agr. Expt. Sta. Res. Bul. 377. 75 pages. Sept., 1943.

Growing animals of four species, including mice, rats, guinea pigs, and rabbits, were treated with thyroactive preparations varying in amounts from relatively large dosages which were toxic to very small dosage which apparently did not affect growth or were so small in amount that it appeared impractical to attempt further reduction in dosage.

The growth rate of mice was consistently and significantly increased by treatment with a rather wide range in dosage (0.01 to 0.04 mg. thyroxine-sodium daily or 0.04 to 0.32 per cent thyroactive iodocasein in the ration while maximum size attained by control and treated animals was unchanged. Feed intake of mice was increased by treatment. The treated animals stored more protein and more body weight per unit of feed consumed than controls while control animals were more efficient in storage of fat and energy.

The effect of feeding thyroactive iodocasein to rats was variable with strain and sex. There was some evidence of slight acceleration of growth in weight of a few females due to feeding thyroactive iodocasein, but for the most part body weight was unaffected or depressed. The nose-anus length

of male rats of one strain (Sprague-Dawley) was increased due to treatment, but was not observed in any of the other animals. Male rats were less tolerant of thyroactive preparations than female.

The growth rate of male guinea pigs was slightly accelerated by mild treatment (0.0025 to 0.0075 per cent thyroactive casein in the ration) for a short period of time, but the same treatment later became toxic with increase in age and arrival of warm weather. Growth rate of female guinea pigs was not affected for a few weeks, after which time treated animals ceased growing and lost weight.

Small amounts of thyroactive casein (0.0025 to 0.02 per cent of the ration) apparently did not affect growth of rabbits while larger amounts caused a depression of growth.

The effect of thyroactive casein on the organ weight of rats was studied. Extremely small dosages given to males did not affect the weight of any of the glands or organs weighed, although the thyroids of treated animals showed histological evidence of inactivity. Larger dosages given to either sex resulted in hypertrophy of heart, liver and kidneys. The effects on other glands was variable with strain and sex of animals.

The thyrotropic potency of the pituitaries of animals was markedly lowered by feeding thyroactive iodocasein.

15 figures, 26 tables, 7 pages of literature references.

J.G.A.

154. **The Mammogenic Hormones of the Anterior Pituitary. II. The Lobule-alveolar Growth Factor.** J. P. MIXNER AND C. W. TURNER. Mo. Agr. Expt. Sta. Res. Bul. 378. 62 pages, illus. Sept., 1943.

1. Mammary lobule-alveolar growth responses were secured in castrate virgin female mice with anterior pituitary materials injected over periods of time ranging from four to ten days. These responses were not very predictable or repeatable on reessay.

2. The simultaneous injection of pituitary preparations and a small amount of estrone greatly reduced the amount of pituitary required to secure alveolar responses and the dosages of pituitary preparations injected were proportional to the per cent positive lobule-alveolar responses secured in groups of assay mice.

3. In the development of an assay method for the mammogenic lobule-alveolar growth factor, a ten-day assay period was found to be optimal.

4. The length of time elapsing between ovariectomy of the assay mice and the beginning of injection affects the mammary response secured; the shorter the time, the greater the mammary response.

5. An assay for the mammogenic lobule-alveolar growth factor was formulated. A mouse unit of this factor was defined as the amount of material required per mouse injected over a ten-day period to obtain minimal lobule-alveolar growth in 50 ± 10 per cent of ten or more castrate nulliparous

female mice when a total of 75 I.U. of estrone is simultaneously injected. Injection of the assay animals should start immediately after ovariectomy unless they are first primed with estrogen for several days preliminary to the start of injections.

6. Estrone in amounts of 40 I.U. to 133 I.U. were found to synergize best with one mg. of progesterone in stimulating mammary lobule-alveolar growth. Greater or smaller amounts of estrone did not give optimum synergism with progesterone.*

7. Progesterone or pregnenolone alone caused lobule-alveolar growth. However, five or six times as much was required as if estrogen was also injected.

8. Although 2400 I.U. of estrone was able to completely inhibit the activity of a mouse unit of progesterone (one mg.), it was unable to inhibit the activity of a mouse unit of a pituitary preparation.

9. As a result of a series of experiments with progesterone, pregnenolone, pituitary extracts and estrogen, it was suggested that estrogen enhances the activity of progesterone and pituitary materials in stimulating mammary lobule-alveolar growth by acting directly on the stromal tissue surrounding the mammary gland producing an increased hyperemia and vascularity associated with an increased permeability of the vascular system. This condition would allow a circulating pituitary mammogen to be maximally effective in causing mammary gland growth.

10. Both estradiol benzoate and diethylstilbestrol were able to substitute for estrone in conjunction with progesterone in enhancing mammary lobule-alveolar growth.

11. Assays of various types of pituitary extracts showed that the mam-mogenic lobule-alveolar growth factor is protein in nature. These assays also indicate that this factor is not identical with lactogen, thyrotropin or gonadotropin.

12. Progesterone, pregnenolone, desoxycorticosterone, dehydroandrosterone, diethylstilbestrol, acetoxy-pregnenolone, and methyl testosterone ranked in the above order in their ability to stimulate mammary lobule-alveolar growth.

13. High environmental temperature inhibited the ability of progesterone and estrone to stimulate mammary lobule-alveolar growth. This same high temperature was unable to inhibit the ability of a pituitary preparation to stimulate lobule-alveolar growth.

14. Thyroxine in suitable amounts increased by about 33% the efficiency of progesterone in stimulating lobule-alveolar growth.

15. Thyroidectomy greatly decreased the efficiency of both progesterone and pituitary preparations in stimulating mammary lobule-alveolar growth.

16. Virgin female goats injected daily with twenty or thirty mg. of progesterone plus 100 or 150 micrograms of diethylstilbestrol, respectively,

for sixty days were stimulated to develop mammary glands similar to that seen in midpregnancy. Twelve days additional treatment with 0.25 mg daily of diethylstilbestrol caused an initiation of secretion in these mammary glands similar to that seen at the time of parturition.

17. The response of virgin female goats to diethylstilbestrol in regard to mammary lobule-alveolar growth was extremely variable. In some cases very slight stimulation of the lobule-alveolar system was effected while in others a considerable development was secured.

18. Lobule-alveolar growth secured with diethylstilbestrol injections in goats was not histologically typical of that seen in normal lactating glands. The alveoli were much larger and less dense than normal lactating alveoli. Abnormal papillae-like structures were seen protruding into the lumina of the alveoli.

19. The over-all picture of mammary gland development as affected by the various endocrine glands was discussed.

20 figures, 18 tables, 6 pages of literature references.

J.G.A.

155. The Effect of Thyroxine and Dinitrophenol on Sperm Metabolism.

HENRY A. LARDY AND PAUL H. PHILLIPS, Dept. Biochem., Univ. Wis. Jour. Biol. Chem., 149, No. 1: 177. 1943.

Dinitrophenol, a substance which stimulates tissue respiration, stimulated both the glycolysis and respiration, in the presence of metabolites, of bull spermatozoa, but it inhibited endogenous respiration. Dinitrophenol inhibited sperm motility and this could be prevented, to some extent, by the addition of glucose, lactate, or pyruvate.

"Thyroxine in 1:75,000 dilution inhibited respiration of bull spermatozoa and stimulated glycolysis. Orthothyroxine, an isomer of low physiological activity, did not significantly affect either glycolysis or respiration."

A.O.C.

MISCELLANEOUS

156. Reducing Hazards of Winter Truck Operation. E. G. QUESNEL, Dir. of Safety, The Borden Co. Milk Dealer, 33, No. 4: 24. Jan., 1944.

The protection of life and the prevention of accidents, particularly under extra hazards of winter driving when streets are icy, snowy, and full of ruts, is of first importance in the operation of milk delivery trucks. The driver is admonished to distribute load evenly, to keep windshield clear, avoid use of brakes, keep out of ruts, shift gears on hills, brake with engine, drive slowly and carefully and check motor combustion and exhaust. Extra precautions should be taken in meeting, following, and passing other cars and trucks.

C.S.T.

ABSTRACTS OF LITERATURE

BOOK REVIEW

157. **Handbook for the Etiology, Diagnosis and Control of Infectious Bovine Mastitis.** IVAL ARTHUR MERCHANT AND R. ALLEN PACKER, Iowa State College. Published by Burgess Publishing Co., Minneapolis, Minn. 66 pages. \$1.25.

The authors of this handbook should be commended for their effort to gather scattered information regarding an important animal disease, sift out a great deal of cumbersome detail and concentrate the important factual matter between the covers of a small handbook.

This work will be a useful addition to the libraries of teachers, students and practitioners. Predisposing influences, bacteriology, diagnosis, control and treatment of the disease are the important subjects discussed. Sufficient detail is included to make the work a useful guide in procedures and techniques.

T.S.S.

BACTERIOLOGY

158. **Effect of Increase in Acidity on Antiseptic Efficiency.** OTTO RAHN AND JEAN E. CONN, N. Y. State Col. of Agr., Cornell Univ., Ithaca, N. Y. Jour. Indus. and Engin. Chem., Indus. Ed., 36, No. 2: 185. Feb., 1944.

Benzoic acid, salicylic acid, and sulfurous acid are nearly a hundred times as efficient antiseptics in strongly acid solutions as they are in neutral solutions. The toxic principle of benzoic and salicylic acids is the undissociated acid molecule. Growth of a wine yeast was completely suppressed when the concentration of undissociated benzoic acid reached 25 mg. per 100 ml. or when 4 mg. of undissociated salicylic acid was present. When sulfur dioxide dissociates in water the HSO_3^- ions inhibit the multiplication of *B. coli* but not of yeast. The rapid death of yeast is brought about by 7 to 8 mg. of undissociated H_2SO_3 per 100 ml.; *B. coli* can tolerate nearly ten times as much.

B.H.W.

BUTTER

159. **Sanitation in Buttermaking.** WENDELL VINCENT. Amer. Butter Rev., 5, No. 8: 238-241. 1943.

The inspector and plant operator are responsible for practices affecting sanitation within the plant as well as with the finished product. Sediment testing of cream is necessary and straining of cream by means of moving cloths rather than by in-line filters should be advised. Use of polluted water

and regard of pasteurization as a cover for poor sanitation are common offenses. Neglect in making repairs by soldering, improper care of stuffing boxes, poor cleansing and rinsing, and insanitary handling of butter scraps result in the production of lower score butter. Cream shipped by cream stations and independent buyers is of much lower quality than that shipped by direct shippers in the ratio of 2:1. While producers have been urged to increase quantity no one has ever urged that this be done at the expense of quality.

P.S.L.

160. **Butter Outlook and Its Relationship to Milk Solids for Ice Cream for 1944.** T. G. STITTS, Chief, Dairy and Poultry Branch, F.D.A., Washington, D. C. *Ice Cream Trade Jour.*, 40, No. 1: 12. Jan., 1944.

A comprehensive picture is drawn by the author of the government's effort to apportion the available milk supply to production of the various dairy products required for civilian, military and lease-lend purposes. Ice cream manufacture is not likely to be further restricted inasmuch as the dairy industry will of necessity "lean heavily" on the ice cream industry to absorb milk solids when the food emergency is over. Butter is likely to be short for the duration but supplies should not become much scarcer than at present. If milk production does not "fall off" too much and if conservation order, F D O 79, works as it should, no coupon rationing of fluid milk will become necessary. A huge amount of cream has been moving into cold storage which tends to dislocate the dairy products program. Much of this should go into butter but it is doubtful if it was stored for that purpose. On the whole, the dairy products program seems to be working quite satisfactorily.

F.J.D.

CHEESE

161. **A Program for Maintaining Cheese Quality.** G. H. WILSTER, Oreg. State Col., Corvallis. *Natl. Butter and Cheese Jour.*, 35, No. 2: 16. Feb., 1944.

The program recommends: grading milk by appearance, sediment test, flavor, methylene blue and curd tests; washing, sterilizing and drying cans; pasteurization of milk; the use of an active starter; limitation of acid development during manufacture to conform to a making period of about 5 hours from setting to milling; strict sanitation in factory maintenance; and careful curing at temperatures selected to bring out the best flavors.

W.V.P.

162. **Did You Ever See a Dream Working?** PAUL MANDT, Olsen Pub. Co., Milwaukee, Wis. *Natl. Butter and Cheese Jour.*, 35, No. 2: 8. Feb., 1944.

A practical version of an ideal cheese factory is described and discussed. This well-equipped plant includes such unusual machinery as an autoclave

for starter cultures, a mechanically refrigerated starter can and glass holding tubes combined with a plate-type, regenerative pasteurizer. Cleanliness is emphasized in plant maintenance and is accomplished by systematic routines, simple instructions and assignment of responsibility to individuals. The plant operates with dry floors during the day. Making operations are run "by the clock" because of starter control methods and pasteurizing efficiency.

W.V.P.

163. Some Causes of Slow Production of Acid During Cheesemaking.

G. H. WILSTER, Oreg. State Col., Corvallis. Natl. Butter and Cheese Jour., 35, No. 3: 10. March, 1944.

The common trouble of a starter "going dead" may be caused by bacteriophage, an unidentified agent which "eats" starter bacteria. Phage may be found in sewage, faeces, pus, and the dust and whey in a cheese factory. It gets into starter by contamination with mist from the whey separator, factory air and dust. The infection may last for months in a factory. Phage is destroyed by treatments which destroy the organisms which it attacks. Prevention of phage infection may be accomplished by disinfecting the factory by spraying, by protection of starter cultures from air-borne phage and by preparation of starter in a separate building designed to exclude phage-laden air.

W.V.P.

164. A Program for Maintaining Cheese Quality. G. H. WILSTER, Oregon

State Col., Corvallis, Oreg. Amer. Butter Rev., 5, No. 9: 270-272, 283-284. 1943.

In view of the precarious situation in the cheese industry due to the manufacture in many cases of a low quality of product the author has outlined and discussed rather completely nine factors important in the improvement of cheese. These are: 1, the grading of milk and rejection of poor quality product; 2, thorough cleansing and proper care of cans after washing; 3, efficient pasteurization of milk; 4, proper use of a good starter; 5, unhurried methods of manufacture with especial attention given to acid development; 6, sanitation; 7, curing at low temperatures and moderate humidity; 8, regulation of moisture and fat content through adequate and regular laboratory control; and, 9, grading as a means of improvement.

P.S.L.

CHEMISTRY

165. The Total Nitrogen Content of Egg Albumin and Other Proteins.

A. C. CHIBNALL, M. W. REES, AND E. F. WILLIAMS, Biochem. Dept., Imperial Col., London, S.W. 7. Biochem. Jour., 37, No. 3: 354-359. Sept., 1943.

With the development of new catalysts for the Kjeldahl nitrogen determination, and especially with the advent of the micro Kjeldahl method, there

has been a tendency to reduce the time required for digesting the sample. The authors feel that this has been done at the expense of accuracy, for the rapid digestion gives lower percentages of nitrogen than those reported by such earlier workers as Osborne and Campbell, as well as their own results with longer digestion periods. "With proteins and protein hydrolysates it is necessary to continue the heating for 8 hours or more after the digest has cleared." In reporting nitrogen determinations on proteins there should be more details of the procedure given, as well as some history of the treatment of the protein or its method of preparation. This may help to intelligently interpret the results.

For casein, prepared by the method of Cohn and Hendry they report 15.73% nitrogen on a moisture-free and ash-free basis. For B-lactoglobulin, which was twice recrystallized, coagulated by heat and washed free from inorganic salts and dried, they report a value of 15.58% nitrogen on a moisture-free and ash-free basis.

Abstractor's note: The term B-lactoglobulin is not frequently used in literature in America. There is a need for a unified system of nomenclature in the field of the milk albumins and globulins. The B-lactoglobulin referred to above is apparently that fraction of the milk albumin which was crystallized by A. D. Palmer. This is the only milk protein reported to have been crystallized to date. A.O.C.

166. **The Dicarboxylic and Basic Amino Acids of Edestin, Egg Albumin and B-Lactoglobulin.** A. C. CHIBNALL, M. W. REES, AND E. F. WILLIAMS, Biochem. Dept., Imperial Col., London, S.W. 7. Biochem. Jour., 37, No. 3: 372-388. Sept., 1943.

A detailed procedure for the estimation of dicarboxylic and basic amino acids is given. The method is admittedly a long one, requiring about 900 working hours, but the authors state that the results are more reliable than those of any method reported heretofore, accounting for all but 1.25% of the total protein nitrogen. The method differs from those usually used in that no reagent is added to the hydrolysate unless it can be quantitatively removed later without carrying with it an appreciable amount of protein.

For B-lactoglobulin the percentages of the total nitrogen as the dicarboxylic acids glutamic and aspartic are given as 13.14% and 6.68% respectively, while the basic amino acids are: arginine 5.95% histidine 2.69% and lysine 12.07% of the total nitrogen. A.O.C.

167. **Oxidative Rancidity in Edible Fats.** L. R. BRYANT, Ont. Agr. Col., Guelph, Ontario. Food in Canada, 4, No. 1: 7. 1944.

Atmospheric oxygen produces a type of rancidity in food fats characterized by changes in color, destruction of the fat-soluble vitamins and the development of off-flavors. The chemical make up determines the suscepti-

bility of various fats to this chemical change. Aside from the fat itself, such factors as temperature, light, ozone, metals or the presence of anti-oxidants accelerate or inhibit the reaction rate. Tests for the susceptibility to, and degree of, oxidation are described. O.R.I.

168. **The Causes, Cures and Methods of Preventing Rancidity.** C. H. CASTELL, Ont. Agr. Col., Guelph, Ontario. Food in Canada, 3, No. 10: 11; 3, No. 11: 11; 3, No. 12: 10. 1943.

This series of three articles deals with fat spoilage from the standpoint of (1) hydrolytic rancidity of non-microbial origin, (2) rancidity and off-flavors produced by yeasts, molds and bacteria of the aerobic group, and (3) off-flavors of a similar character produced by the butyric-acid-forming anaerobes. In the first of these, the chemical make-up of fats is described with stress being placed on strong odors and flavors possessed by some of the free fatty acids. The sources and characteristics of the lipases are discussed and methods of measuring rancidity and the lipase content of foods described. Particular attention is given to milk and dairy products.

In the second article dealing with microbial agencies causing food spoilage it is pointed out that oxidative rancidity is often an important secondary reaction occurring after the fat has first been attacked by bacteria. Yeasts are very rarely lipolytic but most molds produce lipase. Fat-splitting bacteria are numerous and widely distributed and many species grow at low temperatures. Members of the *Alcaligines*, *Aerobacter*, *Achromobacter*, *Pseudomonas* and *Serratia* genera are of greatest importance. Some of the newer methods whereby organisms of this group may be identified or counted are described.

Butyric-acid-forming anaerobes produce a type of rancidity in some foods entirely unrelated to the fat content. These organisms are strict anaerobes, and produce heat-resistant spores. Butyric acid is one of the chief products produced when carbohydrates are fermented. Relatively few species grow in an acid medium or at low temperatures. Culturing and counting require the use of either an anaerobic jar or growth in a corn-liver or cereal grass medium. Dairy products, particularly some European varieties of cheese, sometimes develop rancid flavors as a result of the growth of organisms of this group. O.R.I.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

169. **Improving Keeping Quality of Dry Whole Milk.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State Col. Milk Plant Monthly, 32, No. 10: 28-29. 1943.

By removal of a large percentage of lecithin through churning and separating out the butter oil and through supercentrifuging the skimmilk,

atmospheric roll dry whole milk of good keeping quality could be made from the recombined product. The possibility of removing some other pro-oxidant than lecithin by the supercentrifuging treatment was suggested by the great improvement in keeping quality noted. The prevalent flavor defect occurring in dry whole milk is of an oxidative type. G.M.T.

FOOD VALUE OF DAIRY PRODUCTS

170. **Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure.** PAUL D. BOYER, ROBERT SPITZER, CURTIS JENSEN, AND PAUL H. PHILLIPS, College of Agr., Univ. of Wis., Madison, Wis. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 16, No. 2: 101. Feb., 1944.

The authors describe a rapid procedure for the extraction and determination of vitamin A and carotene in milk. Two volumes of milk mixed with 3 volumes of alcoholic potassium hydroxide are allowed to stand for 3 hours at room temperature. The mixture is then extracted twice with ether and the vitamin A and carotene are determined by means of the Carr-Price reaction and with the aid of an Evelyn photoelectric colorimeter. The new procedure was compared to the longer procedure of Olsen, Hegsted, and Peterson. The comparative analyses showed that with a single ether extraction the new method occasionally gave low results for vitamin A. The double extraction procedure which was described gave good checks with both pasteurized and raw milks from cows of various breeds. The carotene determination as given in the procedure is a measure of the total carotenoids in the milk. B.H.W.

171. **Determination of Vitamin A and Carotenoids in Butterfat. Comparison of Direct Spectrophotometry with Filter Photometry and Use of the Antimony Trichloride Reaction.** F. P. ZCHEILE, H. A. NASH, R. L. HENRY, AND L. F. GREEN, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. *Jour. Indus. and Engin. Chem., Analyt. Ed.*, 16, No. 2: 83. Feb., 1944.

The data reported were obtained during a comparative study of methods for the determination of vitamin A and carotenoids in butter by the Technical Committee on Vitamin A Researches in cooperation with the National Cooperative Project on the Vitamin A Potency of Market Butters. Six representative samples of butterfat from sweet cream were prepared and analyzed by seven collaborators. This paper compares the results of the direct spectroscopic method used by the authors and the colorimetry methods used by the other collaborators. Comparison of the carotenoid content of the butterfats determined by the different methods showed that from the total of 42 determinations, only 5 deviated from their corresponding means

by more than 7%, the maximum deviation being 13.3%. The direct spectrophotometry results had both the smallest mean absolute deviation and the smallest maximum deviation. Comparison of the determinations of vitamin A contents showed the results from direct spectrophotometry to have mean absolute and maximum deviations two-thirds as great as the over-all averages. The antimony trichloride reaction was considered the preferred physico-chemical method available for butters containing azo dyes.

B.H.W.

172. **Some Experiments on the Possible Relationship between Vitamin C and Calcification.** GEOFFREY H. BOURNE, Univ. Lab. of Physiol., Oxford. *Jour. Physiol.*, 102, No. 3: 319. Dec., 1943.

"Bone salt does not appear to be deposited (except in severe scurvy) until there is an adequate fibrous matrix to receive it. The production of the fibrous matrix of bone and the deposition of bone salt are therefore simultaneous processes. It would seem that as long as there is sufficient vitamin C to produce matrix then that matrix will be calcified. The function of vitamin C in bone formation appears to be to facilitate the production, not just of bone matrix, but of bone matrix impregnated with phosphatase. There is no evidence that vitamin C can be regarded as a coenzyme of phosphatase in calcificatory processes. The apparent reduction of phosphatase activity in scurvy is therefore probably due to a reduction in the amount of bone matrix produced.

"That it is actually vitamin C and not some associated impurity that is responsible for this is suggested by the fact that the administration of vitamin P (citric) and sodium citrate did not result in the formation of more osteoid trabeculae or the deposition of more bone salt than vitamin C alone."

D.E.

HERD MANAGEMENT

173. **Preparing the Cow for Milking.** W. E. PETERSEN, Prof. of Dairy Husbandry, Univ. of Minn. *Milk Plant Monthly*, 32, No. 12: 26-27. 1943.

"Letting down" of milk is the result of a positive act causing tiny muscle cells to contract thus squeezing the milk out of the alveoli where it is made. This is a reflex act, spontaneous to a stimulus, which causes the pituitary gland to secrete a hormone (oxytocin) into the blood by which it is carried to the mammary gland.

The following fundamentals about the response and action of this reflex furnish the basis for several recommended practices: 1. Response to the stimulus is interfered with by any condition that distracts the cow. 2. Once the "let down" has occurred, it is effective for but a short period of time.

3. Cows may become conditioned to let down their milk to a number of different stimuli. 4. The way a cow responds to milking is determined largely by training. 5. About 45 seconds are required from the application of the stimulus to the "let down" of the milk.

The following 11 rules are based on these fundamentals: 1. Handle heifers carefully when they are first milked. 2. Avoid the unusual during milking. 3. Do not treat the cow roughly at any time. 4. The milker must be a person who does not arouse the cow's suspicion. 5. Do not wash or massage the udders or stimulate cows in other ways to let down their milk before milking is to begin. 6. Milk those cows first which let down their milk in response to preparations for milking. 7. Milk rapidly. 8. Do not practice prolonged stripping. 9. Operate milking machines according to the manufacturers' directions. 10. Do not leave the milking machine on the cow after the milk has ceased flowing. 11. Develop the technique of knowing by feel when the gland has been emptied of milk.

For the best operation of milking machine the following 4 points must be observed: 1. Stimulate the cow to let down her milk about one minute before attaching the machine. 2. Operate the machine according to the manufacturer's directions. 3. As soon as the teat cups begin crawling upward begin stripping by tugging downward with sufficient force to lower the teat cups part way down on the teat and at the same time stroke each quarter downward in succession with the free hand. 4. Remove the machine as soon as milk ceases flowing.

G.M.T.

ICE CREAM

174. **Ice Cream with the A.E.F.** S/Sgt. J. A. RABUFFO, E.T.O., England.
Ice Cream Trade Jour., 40, No. 2: 24. Feb., 1944.

After mentioning the shipment of ice cream freezers, fountains, ice cream mix and accessories which have been authorized for distribution and installation in the European Theater of Operations to supply Uncle Sam's soldiers with fountain products which they have greatly missed, "G. I. Joe" tells of the ingenuity of the cooks and "K.P.'s" in improvising ice cream freezers and ice cream mixes under all manner of circumstances. He admits that results rarely compare favorably with the ice cream to which they have been accustomed but are far better than none. In many cases arrangements have been made with local ice cream makers (who no longer make) to use freezing equipment, if the commissaries are able to provide some form of mix ingredients. "G. I. Joe" presents one cook's closely guarded secret formula, revealed in a weak moment, as being composed of: evaporated milk, powdered milk, water, sugar, powdered eggs, corn starch and a little salt. The concoction is brought to a boil, cooled and frozen.

F.J.D.

175. **Pooling Cabinet Service.** W. H. SNEATH, William Neilson, Ltd., Toronto, Canada. *Ice Cream Trade Jour.*, 40, No. 2: 30. Feb., 1944.

A pooled service for checking, adjusting and repairing refrigerated cabinets belonging to Canadian ice cream manufacturers is explained. The system was applied throughout the country by establishing zones, and all prices for parts, mileage rates, hourly wages, etc., were made uniform for each zone. One company was made responsible for all services in each zone and while the yearly records have not been compiled and summarized, there is little question but what tremendous savings have been achieved in gasoline, tires, mobile equipment, manpower and in the avoidance of breakdown. It is emphasized that successful operation of such a cooperative service can only be obtained through the confidence and cooperation of the entire personnel as well as the organizations involved. F.J.D.

176. **Sherbets—What About Their Future?** V. M. RABUFFO, *Ice Cream Trade Jour.*, New York City. *Ice Cream Trade Jour.*, 40, No. 2: 16. Feb., 1944.

With an estimated production of 75,000,000 gallons of sherbets and ices forecast for 1944, the author reviews the experiences of the ice cream industry during 1943, pointing out the errors made in "forcing" these products on dealers as a result of FDO-8 rather than preparing the way by a "selling" campaign. Opinions and experiences are cited and the potentialities of sherbets and ices in the post war picture are stressed. It is pointed out that much progress has lately been made in improving the quality of sherbets and ices and since there is a strong possibility of continued rationing of milk solids, for a period of time after the war, it is suggested that the industry should attempt to correct its recent error by engaging in an extensive effort to sell consumers on the "goodness" and palatability of these milk-solids-sparing products. Sherbets and ices play too vital a role and represent too large a volume to be neglected. The situation is a challenge to the ice cream industry. F.J.D.

177. **The Bacterial Content of Shell Eggs.** C. K. JOHNS, *Bact. and Dairy Research*, Dom. Dept. of Agr., Ottawa, Canada. *Food in Canada*, 3, No. 12: 15. 1943.

Bacteria count limits were incorporated into the 1943 Canada-Great Britain dried egg contracts. To learn whether or not shell eggs contribute materially to the count of the powder, bacterial numbers were determined on whole eggs by smearing 1/2000 g. loopfuls of mixed egg on tryptone glucose extract milk agar slants. Ninety per cent of the 348 eggs examined gave counts under 10,000 per g. with only one egg in excess of 500,000 per g. The eggs were all 2 months old when examined. O.R.I.

178. Concrete in Ice Cream Plants. LAWRENCE FLYNN. Ice Cream Field, 43, No. 1. Jan., 1944.

Floors which give the best service in food plants must have a top surface which wears well and resists the attacks of fruit juices, sugar solutions, and lactic acid, the author states.

Floors with porous surfaces are much more readily affected by acids and sugar solutions than are those with impervious surfaces. Special treatments can be employed to make porous surfaces practically impervious.

Dairy and ice cream plant floors are subject to hard usage and should be heavy duty concrete floors. The following basic principles are listed as essential in producing wear-resistant floor finishes: 1. Use only suitable, clean materials. 2. Use not in excess of $4\frac{1}{2}$ –5 gallons of mixing water per sack of cement. 3. Avoid "segregation" resulting in free water and fine material on top surface. 4. Keep concrete damp as long as possible. At least a week is required for normal Portland cement and three days when high early Portland cement is employed.

The importance of selecting suitable aggregate material and the use of proper proportions of ingredients as well as employing the proper methods and procedures are stressed. To avoid cracking of floor surfaces the author suggests the use of light wire mesh, 4×4 inch, No. 10 gauge wire weighing 31 pounds per 100 sq. ft. near the middle of the top or wearing course, and to avoid severe wear it is considered important to have as much coarse aggregate as possible near the surface.

Two methods are described for treating floor surfaces in order to make them impervious. One treatment consists in the application of warm linseed oil, Chinawood oil, or soy bean oil. To assist penetration the first coat should be thin (equal parts of oil and turpentine or other thinner). A second application can be given with a greater proportion of oil to thinner after the first coat has dried. A second method is the application of paraffin. It should have a melting point of 150° F. and should be applied as a paste prepared by melting 4 parts paraffin with 1 part turpentine and 16 parts toluol. Apply with brush and allow to penetrate 24 hours, keeping the floor warm; then polish with polishing machine.

Directions are also given for re-surfacing old floors after chipping away the old concrete to a depth of 1 inch.

W.C.C.

MILK

179. Influence of Temperature in Open and Closed Truck Hauling.

C. M. PESCK, Dairy and Food Dept., Minneapolis, Minn. Amer. Butter Rev., 5, No. 9: 274–276. 1944.

Roof temperature under the same conditions was 16° lower on a cab made from aluminum-painted wood than on black iron. During each stop

roof temperature increased 6°. Cans of cold water and cream appreciably affected the temperature in the truck, lowering it to 70° when the outside temperature at the start was 92°, and 97° at the end of the trip.

With an open truck and cans covered with a canvas the temperature on the floor of the truck was 85° when the cans were first loaded and 88° on arrival at the creamery. Outside temperature was 86° at the start of the trip, 94° at the end. Temperature varied with the wind direction and did not steadily decrease as in the closed truck. Acidity of the cream sharply increased in the canvas-covered open truck. In the original article an accompanying table gives detailed results.

P.S.L.

180. **Testing Homogenized Milk.** S. T. COULTER, Univ. of Minn., Minneapolis, Minn. *Amer. Milk Rev.*, 5, No. 12: 382. 1943.

The method previously proposed by J. C. Marquardt of the New York (Geneva) Agricultural Experiment Station was given several trials at the Minnesota Dairy Department and gave fairly satisfactory results. The method developed is one of modifying the ordinary Babcock procedure.

P.S.L.

181. **Bad Flavors in Milk.** E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. *Amer. Milk Rev.*, 5, Nos. 11 and 12: 338-346, and 372-385. 1943.

This article combines in an interesting and helpful manner the experimental work of many writers as regards flavors that may occur in milk, together with a discussion of conditions favoring their presence and controlling the degree to which they are present. Those discussed more fully are feed, rancid, lipolytic, those due to fat content and to treatment of milk, as homogenization, oxidized, metallic, cooked, disinfectant, and absorbed flavors.

P.S.L.

182. **Milk—Dairy Products Problems.** LELAND SPENCER, Cornell Univ., Ithaca, N. Y. *Amer. Milk Rev.*, 5, No. 9: 276-282. 1943.

More milk has been produced yearly than on the average for 1935-9 but demand has been much greater than production. The military has taken 19% of the production ordinarily available. As compared with 1935-9, consumption by civilians was lower in 1942 by 7% but up 10% for fluid milk, 13% for canned milk, 14% for cheese, and 67% for ice cream. Besides these demands there are those of the export market. Price ceilings and shortage of labor and feed have added to production difficulties. Price regulations have caused in many cases price maladjustments that become disruptive. To cushion to as great extent as possible the effects of the probable shortage this fall the author suggests six remedies: 1, adjustment of price ceilings in markets operating at a disadvantage; 2, confine shipment of milk

within 500 mile distances; 3, grant priority to milk within its own milk shed; 4, ban all sales of cream if necessary; 5, limit fluid milk sales if necessary; 6, ration milk but only if absolutely necessary and as a last resort. P.S.L.

183. **A Portable Resazurin Outfit.** J. G. DAVIS, Natl. Inst. for Res. in Dairying, Shinfield, Reading. *The Milk Indus.*, 24, No. 6: 47. Dec., 1943.

A portable test kit has been developed for the application of the resazurin test in the field. The outfit is essentially designed for use on the farm and in small dairies. A picture of the apparatus and carrying case is shown and list of items contained in the apparatus is given.

It is advantageous to use the portable resazurin outfit directly after milking as positive results on freshly-drawn milk indicate the presence of body cells or a mastitis condition. The more severe the mastitis the quicker is the dye reduced or changed from blue to pink. It is also advantageous to run the resazurin test on the mixed herd milk as it comes into the plant, for resazurin reduction in such milk would probably be due to bacteria.

For quick resazurin tests on the farm the sample can be milked directly into the test tubes after removing the foremilk. It is recommended that individual quarters be tested. The rennet test is recommended for use in conjunction with the resazurin test. The methods for performing these tests are outlined.

Very bad samples will reduce resazurin rapidly and fail to clot with rennet. If a sample clots slowly with rennet but does not reduce resazurin, it may indicate a past history of mastitis. A change of color of resazurin without slow clotting may indicate an incipient infection which should receive treatment at once.

The following advantages of testing cows for abnormal milk are listed:

- Economic advantages. Infected cows are inefficient converters of food to milk.
- Danger of low solids-not-fat. The writer claims that the resazurin-rennet test can be correlated closely with solids-not-fat content.
- Danger of infecting other cows. The resazurin test may pick out bad cases of infection which may be segregated or disposed of or milked last.
- Effect on bacterial count. Abnormal milk usually increases bacterial count of mixed milk from herd.
- Effect of abnormal milk on manufacture of milk products. Abnormal milk produces weak body and slow starter in cheese manufacture.

The test can be used to good advantage in checking animals that are being purchased for addition to the herd.

H.P.

184. **Interpreting Bacterial Counts to Producers.** O. A. GHIGOILE, Chief, Bur. of Dairy Serv., Calif. State Dept. of Agr. *Milk Plant Monthly*, 32, No. 10: 26-27. 1943.

The responsibility of producing good milk rests with the producer, who

should be instructed in a non-technical language about bacteria, their mode of entry into milk, their rate of multiplication, and the cleanliness and care of dairy equipment. The manner in which the producer is approached will do much in bringing about cooperation in producing a better grade milk. Once the producer realizes and accepts his full responsibility in producing high quality milk with a low bacterial count, he will be proud of his accomplishment.

G.M.T.

- 185. How to Prevent and Remove Milk Deposits.** LEWIS SHERE, Pres.,
The Diversey Corp. Milk Plant Monthly, 32, No. 11: 32, 34, 39, 40.
1943.

Milk stone is a complex homogeneous mixture of organic and inorganic substances which adhere tenaciously to dairy equipment. It is unsightly, may cause off-flavors, interferes with heat transfer and is a source of high bacteria counts. The amount and composition of milk stone is influenced by: 1, the speed with which the milk flows through the equipment; 2, the final temperature to which the milk is heated; 3, the amount of milk handled; 4, type of dairy product handled; 5, type of equipment used; 6, hardness of the water; 7, nature of cleaners and sterilizers; and 8, time of rinsing.

Milk stone formation may be minimized by: 1. Rinsing the equipment with cold water; 2. Cleaning equipment thoroughly every day; 3. Rinsing the cleaning solution from the equipment; 4. Using chlorine sterilizers rather than heat; 5. Preventing corrosion of equipment.

Accumulated milk stone may be removed effectively and efficiently by using an acid-type product, following the manufacturer's directions. Precautions should be taken that the equipment is not damaged.

G.M.T.

- 186. Interpreting Bacterial Counts to Producers.** WALTER D. TIEDEMAN,
Chief of Bur. of Milk Sanitation, N. Y. State Dept. of Health. Milk
Plant Monthly, 32, No. 10: 25-26. 1943.

Bacterial examination of milk is done primarily to improve the quality of the raw milk supply. Interpretations of the bacteria count should be made in a language the producers understand by one who appreciates the limitations of the laboratory tests. Since there is no advantage in reporting figures, the reporting of the following classes of bacteria is suggested: class 1—200,000 or less; class 2—from 200,000 to 1,000,000; class 3—over 1,000,000.

Milk falling in class 1 is satisfactory; that in class 2 needs improvement; while that in class 3 calls for immediate and definite action.

A similar classification of cell counts is suggested as follows: cells + = 500,000 or less per ml.; cells ++ = from 500,000 to 5,000,000; cells +++ = over 5,000,000.

A two-plus cell count might be interpreted as indicating the presence of mastitis in the herd, while a three-plus cell count is more definitely an indication of mastitis particularly when accompanied by long chain streptococci. The technician should help the dairyman by observing the type of bacteria and suggesting their significance. Repeating counts after unsatisfactory reports have been reported to see whether the report has been translated into action is good practice.

G.M.T.

PHYSIOLOGY

187. **The Blood Volume of Normal Animals.** F. C. COURTICE. *Jour. Physiol.*, 102, No. 3: 290. Dec., 1943.

The blood volume of 30 goats has been estimated with the blue dye T-1824. The mean plasma volume was 53 cc. per kg. body weight and blood volume was 70 cc. per kg. The blood volume of goats as well as that of rabbits, dogs and horses is proportional to the body weight and not to surface area. The effect on blood volume of stage of lactation or productivity is not mentioned. The author does state that the blood volume of four highly trained greyhounds was much higher due to a higher cell volume. The blood volume depends upon the bulk of the animal tissue, especially muscle, and not upon the rate of metabolism.

D.E.

188. **Lipolysis and Fat Absorption.** A. C. FRAZER, *Physiol. Dept., St. Mary's Hosp. Med. School, London, and the Pharmacol. Dept., Univ. of London.* *Jour. Physiol.*, 102, No. 3: 329. Dec., 1943.

The ingestion of neutral fat normally leads to a characteristic appearance of the intestinal cells, to milkyness of the lacteals, to a marked systemic lipemia and to deposition of fat in the fat depots. The addition of potent lipase to the ingested neutral fat causes small instead of large granules to appear in the intestinal cells, the lacteals remain almost clear, the systemic blood shows but a slight lipemia, and the deposition of the fats is much decreased. The portal blood and liver, which show only slight changes after neutral fat ingestion, exhibit marked lipemia and deposition respectively if lipase is added to the neutral fat. The results following the ingestion of neutral fat and lipase are thus similar to those seen after the administration of fatty acid. It is possible to suppress almost completely the post-absorptive systemic lipemia by the addition of lipase to the standard fat-containing meal. Lipolysis should be regarded as a determining factor in the fate of absorbed fat and possibly as a means of providing essential raw materials for the synthesis of lecithin and the formation of soaps.

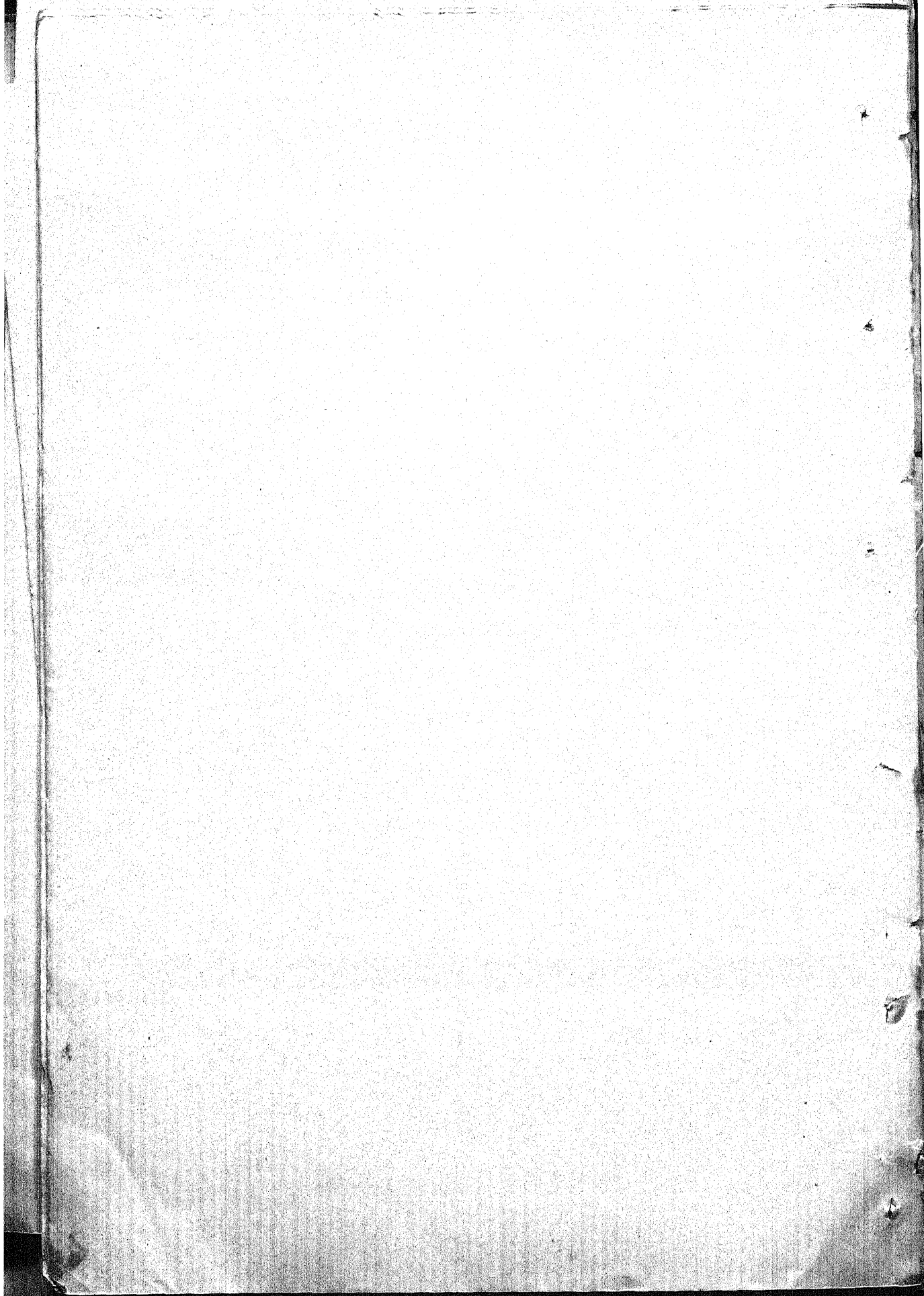
The complete inhibition of lipolysis by a long chain sulphate, sodium cetyl sulphate, in rats, does not prevent triglyceride absorption.

D.E.

189. **Differentiation in the Absorption of Olive Oil and Oleic Acid in the Rat.** A. C. FRAZER, Physiol. Dept., St. Mary's Hosp. Med. School, London, and the Pharmacol. Dept., Univ. of Birmingham. *Jour. Physiol.*, 102, No. 3: 306. Dec., 1943.

According to the author's view, lipolysis is only partial in the intestinal tract of the adult rat, and hydrolysis of the triglyceride molecule is not regarded as an essential preliminary to its absorption. Fatty acid passes by the portal vein to the liver, while neutral fat goes by the lymphatic route to the systemic blood and thence to the main fat depots to be stored for future use. Stained fatty acids fed over a period of 10 days result in no staining of these areas, but rather, appear in the liver. The degree of lipolysis is, thus, a determining factor in the immediate fate of absorbed fat.

D.E.



ABSTRACTS OF LITERATURE

BACTERIOLOGY

190. **Simple Multiple-Loop Method Speeds Bacterial Counts.** ANDREW MOLDAVAN, Pure Milk Co., Montreal, Canada. *Food Indus.*, 15, No. 6: 56. June, 1943.

The author states that plate counts are time-consuming. In order to simplify bacteria estimates a modified Myers-Spence procedure is described.

It involves using a loop and melted cooled nutrient agar. Thus the operator is given a chance to do many determinations with a minimum of effort.

The apparatus is described. Loop and pipette accuracy is discussed, including a method for calibrating the loop with a 0.01-ml. pipette.

J.C.M.

191. **Research Finds Substitute for Bacteriological Agar.** W. E. BAIER AND T. O. MANCHESTER, Ontario. *Food Indus.*, 15, No. 7: 94. July, 1943.

A shortage of agar has caused investigators to search for a substitute for bacteriological agar. As a result, a pectinous material, sodium ammonium pectate, has been used successfully in some of the most commonly employed solid media.

Culture media for plates and slants, particularly counting plates considered here, demand a gel-producing constituent which meets very exacting specifications. Other gel materials have been suggested and some have been used in special applications, but agar has been and still remains the standard gel-forming agent for bacteriological culture media.

The article gives formulae for making the substitutes for agar for milk work. Details are given for using the substitute for agar.

J.C.M.

CHEESE

192. **New Way to Dehydrate Cheese.** GEORGE P. SANDERS, U.S.D.A., Washington, D. C. *Food Indus.*, 15, No. 10: 80. Oct., 1943.

American cheddar cheese usually contains more than 33% water, removal of which would reduce the weight by at least one-third, and would also make it possible to compress the cheese into a smaller volume. These savings are important under wartime shipping and storage conditions, and therefore the commercial dehydration of cheese has been undertaken.

Under the new method, which is applicable to any type of hard cheese, only properly cured cheese, selected with particular attention to flavor, and

thoroughly cleaned, should be used. This is blended as in the manufacture of process cheese. The subsurface portion of the rind, if clean and edible, can be used in the blend.

The author gives details of drying and compressing. Uses and quality as affecting the process are discussed. The author feels that the laboratory successes deem a commercial trial as being desirable.

J.C.M.

CHEMISTRY

193. **Chromatographic Determination of Carotene in Alfalfa.** L. W. CHARKEY AND H. S. WILGUS, JR., Colo. Agr. Expt. Sta., Fort Collins, Colo. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 3: 184. Mar., 1944.

Three important factors may cause errors in the determination of carotene in plant tissues. There may be oxidative losses of carotene, errors due to incomplete extraction from the tissue or incomplete separation of carotenes from other pigments. The chromatographic method described in this report, supported by experimental data, avoids these sources of error. The method includes an enzyme inactivation and sample storage procedure, making possible the collection and preparation of large numbers of samples on fixed dates. The chromatographic technique was modified by converting the adsorption column to an adsorption filter which avoided losses of adsorbed carotene.

B.H.W.

194. **Yeast Microbiological Methods for Determination of Vitamins. Pantothenic Acid.** LAWRENCE ATKIN, WILLIAM L. WILLIAMS, ALFRED S. SCHULTZ, AND CHARLES N. FREY, Fleischmann Labs., Standard Brands, Inc., New York, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 1: 67. Jan., 1944.

Pantothenate is determined by adding an extract of the unknown to 5 ml. of basal pantothenic acid-free medium in a test tube, heating, then cooling and inoculating the tubes with 1 ml. of the yeast, *Saccharomyces carlsbergensis*. The tubes are shaken at 30° C. for 16 to 18 hours and the yeast growth is estimated by turbidimetric measurements made directly on the tubes with the photoelectric colorimeter. The basal medium contains ammonium sulfate as a nitrogen source and in addition sufficient asparagine to prevent interference due to B-alanine. Extracts of substances to be assayed are prepared by aqueous extraction under pressure (15 pounds for 15 minutes) at pH 5.6 to 5.7, by enzyme digestion at the same pH, or by enzyme digestion followed by aqueous extraction (15 pounds for 15 minutes). The choice of extraction method depends upon the substance, since some have pantothenate in a bound form whereas others do not. The results

of assays of a number of substances including pasteurized milk, dry skim milk and whey compare favorably with results obtained by other methods.

B.H.W.

195. Quantitative Determination of d-Galactose by Selective Fermentation with Special Reference to Plant Mucilages. LOUIS E. WISE AND JOHN W. APPLING, Inst. of Paper Chemistry, Appleton, Wis. Jour. Indus. and Engin. Chem., Analyt. Ed., 18, No. 1: 28. Jan., 1944.

A method is described which permits the determination of small amounts of d-galactose in the presence of mannose, glucose, fructose, xylose, arabinose and glucuronic acid with an accuracy of 92% to 98%. It depends on differential fermentations with two yeasts, *Saccharomyces carlsbergensis* which ferments galactose and *S. bayanus* which does not ferment galactose. The yeasts have little effect on xylose, arabinose or glucuronic acid. The reducing values of galactose, mannose and d-glucurone were determined by the Munson-Walker method. The fermentation techniques were successfully applied to the hydrolysis products of pure lactose and certain plant mucilages.

B.H.W.

196. Determination of Vitamin A and Carotenoids in Butterfat. Spectroscopic Characteristics of Butterfat Fractions and Problems Involved in Biological Interpretations. F. P. ZSCHEILE, R. L. HENRY, J. W. WHITE, JR., H. A. NASH, C. L. SHREWSBURY, AND S. M. HAUGE, Purdue Univ. Agr. Expt. Sta., Lafayette, Indiana. Jour. Indus. and Engin. Chem., Analyt. Ed., 16, No. 3: 190. Mar., 1944.

Butterfat samples produced under different dietary conditions were studied by the direct spectroscopic method. Total carotenoids were estimated and ultraviolet measurements were made on the unsaponified fraction. Curves of the total carotenoids and of the carotene fraction from a series of butters were compared with that of β carotene. Corresponding curves of the unsaponifiable fraction in the ultraviolet region were compared with that of vitamin A. Effects of clarification, adsorption, acid extraction, and freezing upon the curves were studied, as well as various factors affecting the reliability of the experimental procedures. No clear-cut relationships could be established as a result of attempts to correlate spectroscopic and biological values. The feed of the cows had a great influence on the nature of the carotenoids present in the butterfat. There should be more extensive purification of the vitamin A fraction for the successful application of direct spectrophotometry to the determination of vitamin A in butterfats.

B.H.W.

197. **Fatty Acid Monoesters of l-Ascorbic and d-Isoascorbic Acids as Antioxidants for Fats and Oils.** R. W. RIEMENSCHNEIDER, J. TURER, P. A. WELLS, AND WALDO C. AULT, Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia, Pa. *Oil and Soap*, 21, No. 2: 47. Feb., 1944.

Fatty acid monoesters of l-ascorbic and d-isoascorbic acids were found to have antioxygenic activity in fats and oils. These substances were found to counteract the deleterious effect of traces of soap on the stability of fats. In combination with either α -tocopherol or phospholipids or both, the ascorbyl monoesters exhibit marked synergistic antioxidant effect. Postulations as to the cause of synergistic phenomena are presented. J.L.H.

198. **The Antioxidant Properties of Nordihydroguaiaretic Acid.** W. O. LUNDBERG, H. O. HALVORSON, AND G. O. BURR, Univ. of Minn., Minneapolis. *Oil and Soap*, 21, No. 2: 33. Feb., 1944.

The antioxidant properties of nordihydroguaiaretic acid in lard are described. This phenolic-type inhibitor was obtained from a common desert plant (*Larrea divaricata*). Nordihydroguaiaretic acid exhibited synergistic action with ascorbic acid but not with a wheat germ concentrate containing 40% of mixed tocopherols. The effectiveness of the antioxidant in stabilizing lard is to some extent carried over into baked products (pie crusts).

J.L.H.

199. **Formation and Decomposition of Peroxides of Unsaturated Fat Esters.** R. F. PASCHKE AND D. H. WHEELER, U. S. Regional Soybean Industrial Products Lab., Urbana, Ill. *Oil and Soap*, 21, No. 2: 52. Feb., 1944.

The influence of temperature on the formation and decomposition of peroxides of unsaturated fat esters (distilled methyl esters of soybean fat acids) was investigated. The curves of decrease of iodine value and increase in peroxide value were found to parallel each other in the early stages of the reaction. As the temperature of oxidation increased above 35° C., the lower was the level at which the curves deviated and the lower the maximum peroxide value attained. At 15° and 35° C. the same maximum of peroxide values was observed, and at this point the value obtained was approximately 30% of what it would be if all the double bonds destroyed were converted to peroxides. The speed of decomposition of peroxides became progressively greater as the degree of oxidation increased. The rate of decomposition or disappearance of peroxide was found to agree best with that of a bimolecular reaction but definite exceptions were observed.

Investigation of reaction time and effect of oxygen on the determination of peroxides by the acetic acid-potassium iodide method, showed that a one-

hour reaction time in the absence of oxygen was necessary, especially on samples of high peroxide content. J.L.H.

200. The Fluorescence of Chlorophyll in Fats in Relation to Rancidity.

C. S. FRENCH AND W. O. LUNDBERG, Univ. of Minn., Minneapolis, Minn. *Oil and Soap*, 21, No. 1: 23. Jan., 1944.

The "chlorophyll value" as a measure of the keeping quality of oil was investigated. According to theory the color change was due to the "quenching" of the chlorophyll fluorescence by the transfer of excitational energy from chlorophyll molecules to acceptor molecules contained in the fat.

It appears that the disappearance of chlorophyll fluorescence in ultraviolet light is due to the absorption of the light by the cottonseed oil and to the intense white fluorescence of the oil itself rather than to a chemical reaction of some constituent of the oil with the excited chlorophyll. The lack of correlation between either the peroxide value or the conventional stability measurements and amount of chlorophyll fluorescence in the fats used makes the "chlorophyll value" test "appear to have doubtful value as a generally applicable test for fat rancidity or stability."

The absorption of near ultraviolet light by oxidized fats may be related to their content of fat peroxides. J.L.H.

201. The Use of Refractive Index Measurements in Fatty Acid Ester Analysis. KARL F. MATTIL AND HERBERT E. LONGENECKER, Univ. of Pittsburgh, Pittsburgh, Pa. *Oil and Soap*, 21, No. 1: 16. Jan., 1944.

The determination of the composition of fats and oils is usually accomplished by the fractional distillation of their mixed methyl esters and the analysis of the separated fractions. When packed columns are used, fractions containing not more than two adjacent homologous components can be obtained. The fractions can then be analyzed for composition by the use of simultaneous equations based on the saponification equivalent, the iodine value and the thiocyanogen value. Two inherent difficulties of the method are, (a) the accuracy with which the analytical constants must be determined and, (b) the small size of certain fractions (0.1 to 0.2 grams).

The linear relationship that exists between the refractive index and the composition of a mixture of adjacent homologous methyl esters makes it possible to use the index as a tool in the calculation of the composition of unknown mixtures. For precise work it is necessary to use a refractometer where the fifth decimal can be estimated and where the temperature can be controlled or known to a few hundredths of a degree. One important advantage of the method is that the analysis can be completed soon after the fraction is taken from the column. J.L.H.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

202. **Compressing Spray-Dried Milk to Save Shipping Space.** B. H. WEBB AND C. F. HUFNAGEL, U. S. Dept. of Agr., Washington, D. C. Food Indus., 15, No. 9: 72. Sept., 1943.

Studies show that as much as 42% of the space occupied by dried milk can be saved by compression, 24% by jolting. Milks subject to different manufacturing conditions, however, may vary widely in their compression characteristics.

Important savings in the amount of shipping space taken up by spray-dried milk can be attained by compression and by jolting or bumping in wartime packaging operations. The savings in space effected by compression may be expected to range from approximately 21% for compression in a small package at a pressure of 500 lb. per square inch to 42% for die compression at 3,200 lb. per square inch. Jolting may be expected to save 11% of the space in a bulk package and 24% in a small package.

The details of both procedures are given, and the advantages listed.

J.C.M.

203. **Removal of Oxidizing Factors Makes Dry Whole Milk Keep.** C. D. DAHLE AND D. V. JOSEPHSON, Penn. State College, State College, Pa. Food Indus., 15, No. 11: 76. Nov., 1943.

Whole milk powder can be treated in processing so as to remove pro-oxidant constituents which make it subject to oxidized or tallowy flavor. The product then has excellent keeping qualities and should satisfactorily meet present requirements.

That whole milk powder develops a stale oxidized flavor after some weeks or months of storage is a fact which has hindered this product from gaining wide acceptance. But the present war has focused attention again on dried whole milk because huge amounts are purchased by the military and lend-lease authorities. No doubt it will prove to be in great demand, even after actual fighting stops.

The authors feel that oxidation of dried whole milk can be improved when better procedures are available to do on a large scale these things which were achieved in a laboratory.

J.C.M.

ICE CREAM

204. **Conserving Milk Solids.** J. H. ERB, The Borden Co., Columbus, Ohio. Ice Cream Trade Jour., 40, No. 3: 20. March, 1944.

It is pointed out that considerable improvement in the quality of "war-time ice cream" is possible, without the substitution of cereal products for lacking milk solids, if the manufacturer makes use of present knowledge and

gives close attention to each step of processing. The following suggestions are made:

Superheated condensed milk should be used rather than unsuperheated, and frozen condensed milk should be avoided. Efficient homogenization is very important. Low solids mixes should be aged 24 hours. The proper quantity of a good stabilizer should be used and it should be handled to get the maximum benefit from it. Mix should be quickly frozen to as stiff a consistency as possible and hardening should be rapid. F.J.D.

205. **Velva Fruit—A New Frozen Fruit Dessert.** H. J. LOEFFLER, Western Regional Res. Lab., U.S.D.A., Albany, Calif. *Ice Cream Trade Jour.*, 40, No. 3: 16. March, 1944.

The author describes a new frozen dessert made from fruit purees. The name "Velva Fruit" is suggested to distinguish the new product from ices and sherbets. Suitable purees can be prepared from a large number of different fruits and some blends. The whole, fleshy, fruit is used in preparing the purees which may then be frozen for distribution to the manufacturer, or they may be used without freezing if kept cold and utilized in a relatively short time.

To the puree is added sugar, gelatin and perhaps citric acid if the fruit is not sufficiently tart. The amount of sugar is varied somewhat with the sweetness of the fruit and about 0.6% of gelatin is usually required. The mixture is frozen in ice cream equipment to approximately 100% overrun. Several formulas are given.

"Velva fruit" differs from ices and sherbets in that the fruit solids and juice account for 60% of the finished product as compared with about 20% where the fruit is used primarily as a flavor. The overrun is also considerably higher but the extra fruit solids prevent excessive fluffiness. "Velva fruit" melts somewhat slower than ice cream but has similar body or dipping qualities at similar temperatures. F.J.D.

206. **An Excess Solids Method for Calculating Ice Cream Mixes.** ALAN LEIGHTON, Bur. of Dairy Indus., Washington, D. C. *Ice Cream Rev.*, 27, No. 3: 24. Oct., 1943.

The author has worked out a variation of the serum point method for calculating ice cream mixes. This method uses the water content of the mix and dairy products, instead of their serum as the reference medium. The method is explained in detail with examples. J.H.E.

207. **Use of Whey Solids in Ice Cream and Sherbets.** ALAN LEIGHTON, Bur. of Dairy Indus., Washington, D. C. *Ice Cream Rev.*, 27, No. 6: 18. Jan., 1944.

Whey solids from Cheddar cheese whey was used in experimental ice cream mixes to replace varying percentages of serum solids. In the case of

10% butterfat and 8% serum solids mixes, 1.6% of whey solids could be substituted with no detriment to quality.

When whey solids were added to mixes to raise the total solids content there was marked improvement in body and texture. No undesirable flavors were noted.

The experiments indicated that whey solids could be used advantageously in wartime ice cream.

Sherbets in which whey solids were used in place of normal milk-solids-not-fat were not noticeably different from the controls.

When using liquid whey it should be pasteurized to inactivate the rennet and thus avoid coagulation.

J.H.E.

208. **Merchandising Ice Cream in War Time.** GEO. W. HENNERICH, Ice Cream Merchandising Inst., Washington, D. C. *Ice Cream Rev.*, 27, No. 3: 70. Oct., 1943.

The idea is expressed that ice cream merchandising is needed now more than ever. A program of merchandising must be carried out that will keep consumer interest in ice cream until consumer demand can again be supplied.

J.H.E.

209. **Quince Seed Extract as an Ice Cream Stabilizer.** GIDEON HADARY AND H. H. SOMMER, Univ. of Wis. *Ice Cream Rev.*, 26, No. 11: 22. June, 1943.

The properties of quince seed water extract as a stabilizer for ice cream have been studied and compared with those of gelatin. Quince stabilizer was found to dissolve readily in ice cream mix at a very wide range of temperatures. The action of quince upon mix viscosity was immediate, in contrast to gelatin which causes an increase with time. Mixes stabilized with 0.032% quince developed much less viscosity than similar mixes stabilized with gelatin.

Quince stabilized ice cream did not melt as uniformly as did those stabilized with gelatin.

At the present time quince seed must be imported and its high cost makes prohibitive its use as an ice cream stabilizer.

J.H.E.

MILK

210. **Safeguarding the Public Milk Supply in War Time.** C. J. BABCOCK, Major, Sanitary Corps, Army Service Forces. *Milk Dealer*, 33, No. 6: 31-32, 72-74. March, 1944.

The problems involved are the same as in peace time but complicated in that loss of enforcement personnel, population shifts, establishment of army camps, lack of processing machinery and availability of milk have been

retarding factors in supplying adequate and safe milk for particular states, cities and towns. Importation of milk from outside sources frequently involved lowered quality standards which the Army, through the Surgeon General and the Quartermaster General charged with safety of Army milk supplies, could not condone. A system of joint control and cooperation between the Army and local control authorities has largely solved the problems involved and assured both Army and civilian personnel of a safe milk supply—made possible by importation of milk from areas of greater production and by extending local milk sheds.

Lack of sufficient control officials has made routine farm inspections difficult if not impossible. Greater emphasis must therefore be placed upon platform inspection and rigid plant control of all processing methods such as daily pasteurization and *B. coli* tests to insure safety. "The dairy industry has not been forced (in the past) by regulations to improve the quality of milk, other than that used for fluid purposes." Herein lies the big problem of obtaining greater satisfactory supplies of milk for fluid use due to general lack of quality in milk used for processing other dairy products—a postwar as well as an immediate problem if the dairy industry is to survive. The need of better butter quality is cited as an example if markets temporarily lost to butter substitutes are to be regained after the war. Local politics, lack of knowledge in control officials and slipshod inspection methods attest to lack of adequate control in many sections—sufficient in some cases to justify complete Army control to the exclusion of local control. The author stresses the need of qualified and specially trained milk control officials in every community and not merely the drafting of milk ordinances whereby a Grade A cap may be placed on a bottle of milk. Tribute is paid to the milk industry for the job it has done in producing a safe milk supply under many handicaps and in creating milk drinking habits in our soldiers. "This milk drinking habit will return with the soldier to the civilian and the per capita consumption of milk will be higher than ever before in history."

C.S.T.

211. Preventing Defects in Bottled Milk and Cream. E. L. FOUTS, Dairy Technol., Agr. Expt. Sta., Gainesville, Fla. *Milk Dealer*, 33, No. 5: 29-30, 62-64. Feb., 1944.

The preventing of defects in bottled milk and cream has always been of vital importance in dairy plants. Under wartime conditions, extra problems are added to dairy plant management and merit closest attention. Listed as added problems for maintenance of quality are: (1) New milk supplies from producers not before qualified to sell milk for fluid consumption. Lack of proper equipment and information on production and handling of such milk needs careful study and cooperation on part of dealers and regulatory officials. (2) The problem of inexperienced employees in the

plant complicate the milk plant's problem. Special instruction and short courses for new workers are suggested as a remedy. (3) Lack of equipment and replacement machinery affect possible defects of milk and cream. Retinning and careful check and repair of all machinery is essential. (4) Less frequent delivery, while advantageous from cost of delivery standpoint, necessitates extra care in providing milk and cream free from flavor and physical defects. Flavor defects to guard against under wartime conditions are (1) oxidized flavor and rancidity, (2) bacteriological contaminations through lack of proper care from cow to container, (3) coliform organisms and (4) poor keeping quality. Eternal vigilance in all processing and handling steps will do much to control and reduce such defects in bottled milk. The defects to avoid in bottled cream of 19% are (1) cream plug, (2) oiling off, (3) feathering and (4) lack of viscosity. To avoid, use fresh cream, handle carefully, heat and cool rapidly, age 24 hours at 35 to 40°, and bottle and sell promptly.

C.S.T.

212. **Postwar Milk Distribution Possibilities.** E. J. MATHER, Exec. Vice-Pres., National Dairy Products Corp. Milk Dealer, 33, No. 35: 33-34. Feb., 1944.

"More people are drinking more milk" than ever before in the United States. This increase has been brought about by the boost given milk consumption by the Army, defense plants, lend-lease and nutrition specialists. Tomorrow's problem is to preserve our present per capita intake of milk. The author stresses need of emphasizing the distinctive flavor of milk as inherent in milk from healthy cows fed correctly and the milk kept free from contamination from the cow to the table. Proper equipment for so producing and handling milk on the farm should therefore be stressed in the dairy equipment supply field. The responsibility of retaining wartime consumptive demands in postwar period is up to the farmer, the supply man and the processor of milk and milk products with economies of present production, procurement, processing and distribution continued and further reductions in manufacturing costs, labor and in equipment to the greatest extent. Then will volume be maintained at lower costs but still at a profit.

C.S.T.

213. **Influence of Cooling Methods on Bacteria in Milk.** T. G. ANDERSON AND JOHN E. NICHOLAS, Penn. State Col. School of Agr. Bul. 454. Sept., 1943.

The milk used in this study was that produced by the college herd. Initial temperature of the milk at the time it was inserted in the cooler varied from 80.5° to 96° F. with an average of 90° F. Temperature measurements of the milk and cooling water were made with thermocouples. When cooling milk with well or spring water it is necessary that the water

temperature not rise higher than 50° F. to produce satisfactory results. Running water should have a temperature of 48° or less. When using ice at least five pounds per gallon of milk are necessary. Ninety per cent of the bacteria in milk are concentrated in the cream layer, two hours after milking. The bacteria remain evenly distributed throughout in the case of homogenized milk. P.H.T.

214. **Milk and Cream Nomograph.** D. S. DAVIS, Wyandotte Chemicals Corp., Wyandotte, Mich. *Food Indus.*, 15, No. 12: 75. Dec., 1943.

This is a graphic interpolation of data on the dependence of solids-not-fat and specific gravity on butterfat percentages in milk and cream. J.C.M.

215. **Morale on the Milk Route.** VIRGIL M. BENEDICT, David's Dairy, Sturgis, Mich. *Milk Dealer*, 33, No. 5: 26, 66-68. Feb., 1944.

Morale is defined as "that something which urges one to give all that he or she has to achieve an objective." The author lists five points, honesty, reliability, business acumen, fairness and pride, as essential on the part of the employers of milk route salesmen if success in selling milk is to be attained. Without these qualities coupled with high morals and character the owner of a milk plant will be unable to build and sustain a high morale in the employee engaged in distributing and selling the company's products—milk and good will. C.S.T.

MISCELLANEOUS

216. **Maintaining Equipment at Peak Efficiency.** B. E. SAVEY, Borden's Dairy and Ice Cream Co., Columbus, Ohio. *Milk Dealer*, 33, No. 6: 104-112. March, 1944.

Proper maintenance reduces repair and requires careful planning, cooperation and a "follow-up" together with rigid adherence to operating manual of each piece of equipment as furnished by the manufacturer and a control system and record of all lubrication, repairs needed or effected, breakdowns or changes made. The factors to consider and stress in maintaining peak efficiency in a dairy plant are (1) proper and careful cleaning of all equipment by all employees; (2) care and repair of pasteurizers; (3) check gauge, packing, oil, valves and drive on homogenizers; (4) check float control, temperatures and scale accumulation on all coolers; (5) pumps should be regularly inspected for packing, gaskets, couplings, covers, pipe connections and motors; (6) clean, oil, check load, keep covered, rewind and overhaul all motors regularly; (7) check wear, grease, gears and replacement parts on all gear boxes; and (8) thoroughly check and inspect all ammonia compressors and condensers in order to avoid a slow-up or breakdown in refrigeration system. Eternal vigilance with all equipment is the keynote

of continued operation under wartime conditions in every dairy products plant. C.S.T.

217. **Aerodynamic Fly Control.** GERALD E. ZICH, N. J. Dept. of Agr., Trenton, N. J. Milk Dealer, 33, No. 6: 29. March, 1944.

A method whereby a current of air, generated by a quarter-horsepower motor blower placed over each doorway, chute or opening is directed downward to curtain the entire opening is described. The air is trained by the angle of a metal flange and then deflected outward at the bottom, blowing the fly or insect back into space instead of into the bottling plant. Diagrams illustrating installation and operation accompany article on this fly control measure said to be effective in eliminating flies in dairies and in milk plants. C.S.T.

218. **Postwar Planning in the Dairy Industry.** MERRILL O. MAUGHAN. Milk Dealer, 33, No. 6: 76-82. March, 1944.

Changing conditions brought about by the war will force the dairy industry to face many new problems. The things to expect as an aftermath of war are listed as follows: 1. Great excesses of goods in all fields with resultant increased competition between industries and between individual concerns. 2. Decreased earnings and reduced working hours, cushioned however by accumulative shortages in many consumer goods. 3. Continued government control of many industries to better effectuate diversion from war to peace status. 4. High consumption for dairy products due to increased emphasis upon nutritional value of all dairy products and upon increased demand of soldiers returning to civilian life "sold" on the value of dairy products. 5. Continued high taxes will prevail. 6. Strong labor unions will continue as a factor in all industries.

"Postwar planning should become postwar preparedness." Suggestions for the dairy industry to adopt now are as follows: 1. Plan for extensive promotion of all dairy products—a National Dairy Council unit in every state and principal market. Plan for both domestic and foreign markets. 2. Plan for extensive research to develop new uses, marketing methods and advertising. 3. Plan for more group action by forming and supporting strong trade associations. 4. Retain good features of war-imposed restrictions and eliminate bad of pre-war practices. 5. Stress quality for all dairy products. 6. Provide for utilization of surplus fluid milk. 7. Return to the realm of economic reality. 8. Plan for greater efficiencies in procurement, labor, processing and sales. 9. Improve our relations with the public. 10. Think in terms of the welfare of the entire industry. "Let's keep faith with each other and in our democratic form of Government." C.S.T.

219. **Some Suggestions for Keeping Those Trucks Rolling.** J. N. BAUMAN, White Motor Co. *Ice Cream Rev.*, 27, No. 6: 21. Jan., 1944.

Three fundamental things must be carried out if trucks are to be kept in efficient operation. These are (1) adequate and correct maintenance, (2) availability of parts when needed, and (3) proper care of truck equipment by the driver.
J.H.E.

220. **Manual of Dairy Detergents and Cleaning Practices.** M. E. PARKER, Beatrice Creamery Co., Chicago, Ill. *Food Indus.*, 15, No. 7: 78. July, 1943.

The attributes and shortcomings of the various types of dairy washing compounds are cited and directions for cleaning cream cans, separators and farm utensils are given.

Effective cleaning of cream transport cans, separator parts and farm utensils is not attained by the mere use of washing compounds and chemical sterilizers. The purpose of any dairy cleaner is to prepare dirt, milk solids and grease for its subsequent detachment by brushing and its final elimination by rinsing.

As with many things about which dairymen have little exact knowledge, many dairy washing compounds sometimes are invested with magical powers. Their function is to remove dirt and grease. This is all any good cleaner can do. Differences in price may be based on the different qualities and combinations of the chemicals used, and other materials added to combat hardness in water or to give the cleaner some special character.

Selecting the right cleaner would be a simple matter if soft water were available everywhere. The degree of hardness in water varies in different parts of the country, even from town to town in some sections.

The various kinds of washing compounds available for dairy cleaning purposes may be classified generally as follows:

1. The alkalies and alkaline salts, such as caustic soda, sodium metasilicate, trisodium phosphate, sodium carbonate and bicarbonate of soda, or various mixtures of such chemicals.
2. The acid materials used for waterstone and milkstone removal, such as inhibited muriatic acid (hydrochloric acid), phosphoric acid, tartaric acid, as well as the new acid cleaners for general cleaning developed within recent years.
3. Natural materials such as wetting agents which rely on neither acidic or alkaline properties in their use.
4. The water-conditioning chemicals commonly referred to as the polyphosphates, which in general have no marked detergent characteristics but do have special properties in the compounding or application of effective cleaner mixtures, nevertheless.

5. Miscellaneous materials such as abrasives, metal cloth, and so forth which are used as mechanical aids with or without cleaning compounds.

J.C.M.

221. **What's Ahead for Private Motor Trucks?** JOSEPH B. EASTMAN
Dir., Office of Defense Transportation. Milk Dealer, 33, No. 5: 31
88-89. Feb., 1944.

Three factors stand out in private motor truck transportation. (1) Dependency of domestic economy and war effort on motor truck transportation. (2) The extent of loss of rubber for truck tires. (3) Competition of private trucks for wartime equipment, repairs and tires. In 1941, 700,000 motor truck units were sold as compared with 100,000 units in 1942 and 1943 but illustrates the complicated problems involved in continuing to operate, maintain and conserve motor truck transportation in the face of increased demands. Office of Defense Transportation's certificate of war necessity records shows a 20% mileage saving in truck operation despite increased demands. Private trucks have accomplished great savings by elimination of extra delivery, by consolidation of routes, greater loads, etc., and have effected in individual cases as much as 40% savings. These savings have not been made by all private trucks and a plan is made for 100% cooperation if private motor trucks are to continue to carry their fair share of wartime hauling.

C.S.T.

222. **Mechanical Treatment Destroys Insects in Foods.** E. S. STATELER
Food Indus., New York City. Food Indus., 15, No. 7: 82. July,
1943.

Under peacetime conditions, the estimated annual loss of \$600,000,000 is an exorbitant toll to pay in food and grain supplies because of insect infestation. Under present conditions, that loss, which may become even greater because of handling, storage and shipping difficulties, is more serious than the mere monetary value involved.

The article contains procedures and precautions which are of value to anyone interested in processing food materials.

J.C.M.

